REMARKS

These remarks are in response to the Office Action mailed November 12, 2008 and are in furtherance of the Interview conducted with Examiner and Supervisor Stucker on May 22, 2009. Claim 15 has been amended. The amendments include subject matter already pending an under examination (see, *e.g.*, claim 24), thus the amendments do not introduce matter that requires a new search.

INTERVIEW

We take this opportunity to thank Examiner Dutt and Supervisor Stucker for the interview conducted on May 22, 2009. In the interview, Applicant, Dr. Feifel, and Applicant's representative, Joseph Baker, discussed the invention and the novelty and non-obviousness of the invention. In particular, Applicant described the unrecognized benefit of NT in modulating serotonin-2A neurotransmission, a novel and non-obvious action of the present compounds and the use of such compounds in treating disorders that had not previously been described as treatable using such compounds.

The Examiner suggested certain amendments may assist in moving the application to allowance (the Examiner is respectfully directed to page 3, the continuation sheet, of the Interview Summary). For example, the Examiner suggested adding the limitation "human" to the claims, as well as amending claim 15 to include "disease or" to be consistent with the remainder of the claims. Applicant has introduced these amendments. Applicant respectfully submit that those of skill in the art use the term "disease" and "disorder" interchangeably to refer to anxiety, depression and bipolar symptoms.

In addition, the Examiner and the Examiner's supervisor appear to believe that the hyperactivity of the animal models used in the disclosure is indicative of anxiety. Animal models of anxiety do not involve increased locomotor activity such as produced in the amphetamine-induced hyperactivity model and, in fact, tend to involve expressions of decreased or restricted activity by an animal. In animals, fear/anxiety results in defensive behaviors, such as freezing or avoiding open, lighted areas in favor of dark, confined (*e.g.* protected) places and these defensive

behaviors are exploited by scientist to study anxiety and investigate potential treatments. Amphetamine-induced increase in locomotion is not, by convention, considered a model of anxiety or depression.

The most common behavioral animal tests of anxiety are elevated plus maze, light-dark box, open field test and freeze behavior (see, *e.g.*, Calabrese, Clin. Rev. Tox., Vol 38, pp. 489-542, 2008, attached hereto as Appendix A).

- Elevated plus maze – consists of two open arms and two enclosed arms. Animals avoid entering the open arms where they are exposed. The degree to which animals spend more time in dark vs. light arms is a quantifiable measure of their anxiety. Drugs that increase proportion of time in light arms are considered to have anti-anxiety potential (see, *e.g.*, Merali *et al.*, Psychopharm., vol. 201, pp. 115-123, 2008, attached hereto as Appendix B).
- Light-dark box – Like elevated plus maze, has light and dark section but instead of a maze, is a box. Drugs that increase proportion of time in light section are considered to have anti-anxiety potential.
- Open field test – Animals movements in a box with four walls and no roof is analyzed. The extent to which animals avoid being in the center area (exposed) and prefer staying close to the walls of the apparatus is a measure of their anxiety. Drugs that increase proportion of time in central section are considered to have anti-anxiety potential (see, *e.g.*, Prut *et al.*, Eur. J. Pharmacol., Vol. 463, pp. 3-33, 2003, attached hereto as Appendix C).
- Freeze behavior – the degree to which animals display freeze behavior (becoming motionless) in response to some stimulus (*e.g.* loud noise) is a measure of animals anxiety. Drugs that reduce freeze behavior are considered to have anti-anxiety potential.
- Fear Potentiated Startle – A neutral stimulus (*e.g.* red light) is paired with mildly painful stimulus (*e.g.* footshock). Animals learn to associate neutral stimulus with pain and neutral stimulus becomes fear conditioned stimulus inducing anxiety. Degree to which sound induced startle response in an animal is increased when sound is paired with fear conditioned stimulus (red light) relative to when sound is presented by itself is a measure of anxiety. Drugs that reverse the potentiation of startle produced by the fear condition

stimulus are considered to have anti-anxiety potential (see, *e.g.*, Shilling *et al.*, Pharmacol. Biochem. And Behav., Vol. 90, pp. 748-752, 2008, attached hereto as Appendix D).

SUMMARY OF THE DISCLOSURE

Different types of brain receptors are known to play a role in the improvement of the symptoms of certain psychiatric disease and disorders including schizophrenia. Stimulation of dopamine receptors for example by dopamine which is released from certain neurons into certain synapses between neurons produces certain symptoms, namely hallucinations (*e.g.*, hearing voices) and paranoid delusions, termed "positive" symptoms of psychosis seen in schizophrenia. Certain drugs including amphetamines are agonists of dopaminergic receptors and stimulate these receptors. Amphetamine for example, stimulates dopamine receptors by causing dopamine containing neurons to release dopamine and by inhibit the reuptake and removal of released dopamine thus extending the duration of dopamine effects on dopamine receptors. In humans amphetamine can induce "positive" symptoms of psychosis similar to that experienced by schizophrenia patients. In animals amphetamine will produce certain behavioral effects such as a stimulation of their locomotor activity and reduces prepulse inhibition (PPI), a normal physiological phenomenon that is a measure of sensorimotor gating. Drugs that reduce the stimulation of dopamine receptors can reverse the effects of amphetamine. Investigators can identify drugs that reduce dopamine receptor stimulation by finding drugs that reverse the effects of amphetamine in animals. Such drugs would be expected to improve positive symptoms of schizophrenia similar to "typical" or "first generation" antipsychotics. Hertel *et al.* and Feifel *et al.* teach and suggest only that NT agonists reduce stimulation of dopamine receptors.

Serotonin receptor are a distinct class of receptors from dopamine receptors and there are several types of serotonin (5-HT) receptors. The serotonin-2A receptors is one type of serotonin receptor implicated in the improvement of certain psychiatric symptoms aside from positive symptoms of schizophrenia. Serotonin released by one neuronal cell binds to and activates 5-HT2A receptors on an adjacent cell causing activation of the neuron. Reduction of stimulation of serotonin-

2A receptors, either alone or in conjunction of reduction of stimulation of dopamine receptors, improves "negative" symptoms of schizophrenia, cognitive deficits seen in schizophrenia and also abnormal mood and anxiety symptoms seen in schizophrenia and other disorders such as bipolar disorder, depression and anxiety. DOI is an agonist of serotonin-2A (5-HT2A). It binds to the serotonin-2A receptor in a way similar to serotonin and produces strong activation of those receptors. Stimulation of serotonin-2A receptors can produce reduction of PPI just like stimulation of dopamine receptors, albeit by a completely different biochemical pathway. Drugs that reduce stimulation of serotonin-2A receptors reverse the PPI effects of DOI but not amphetamine. Similarly drugs that only block dopamine receptors reverse PPI effects produced by amphetamine but not DOI. Investigators can identify drugs that reduce serotonin-2A receptor stimulation by finding drugs that reverse the effects of DOI in animals. Such drugs would be expected to improve negative symptoms and cognitive deficits of schizophrenia and to improve symptoms of many other disorders such as depression, bipolar disorder and anxiety.
This is what is demonstrated by the present application, *i.e.*, it was demonstrated that NT agonists such as NT69L and PD149163 reverse DOI-induced PPI reduction. This was a property of NT agonists not previously taught or suggest in the art.

Typical antipsychotics reverse amphetamine but not DOI induced PPI reduction. This is consistent with their known ability to strongly bind and block dopamine receptors but not serotonin-2A receptors. Typical antipsychotics are efficacious for positive symptoms of schizophrenia but not highly efficacious for negative symptoms, cognitive deficits of schizophrenia or for bipolar disorder, depression or anxiety. In contrast, "atypical" or second generation antipsychotics are known to bind to and inhibit both dopamine and serotonin-2A receptors and these drugs, unlike typical antipsychotics, can reverse both amphetamine and DOI induced PPI effects in animals. Atypical antipsychotics are used to treat a wide variety of neuropsychiatric symptoms including negative and cognitive deficits of schizophrenia, bipolar disorder, depression and anxiety and this is mostly made possible by the fact that they modulate serotonin-2A receptors.

The ability of drug to inhibit alpha-1 receptors is also thought to be beneficial in treating psychiatric symptoms. However, this the therapeutic benefit of inhibiting

alpha-1 receptors is thought to only be exhibited in the presence of existing inhibition of either serotonin-2A or dopamine receptor inhibition (augmentation effect). Investigators can test the ability of a drug to inhibit alpha-1 receptor activation by testing its ability to reverse PPI disruption produced by an alpha-1 agonist such a cirazoline. The ability of NT agonists to inhibit alpha-1 receptor activation is what was demonstrated in the disclosure in addition to serotonin-2A receptor inhibition. This was not taught or suggested by the cited references. The ability of NT agonists to inhibit alpha-1 activation in addition to inhibition of activation of serotonin-2A and dopamine makes NT agonists highly auspicious for the treatment of a wider variety of psychiatric symptoms.

Typical and atypical antipsychotics reduce activation of receptors, be it dopamine, serotonin-2A or alpha-1 by binding to those receptors. NT agonists are able to reduce activation of these receptors despite the fact that they do not bind any of these receptors. It is thought that they are able to do this by activating NT receptors which in turn shut down the pathways in which dopamine, serotonin and alpha receptors act. Thus NT agonists represent a novel mechanism by which to reduce activation of these receptors.

One major problem with atypical antipsychotics is that they frequently produce weight gain, diabetes and elevated cholesterol. NT agonists, on the other hand, reduce food intake and weight. Thus NT agonists may represent a way to treat many of the psychiatric disorders currently being treated with atypical antipsychotics with much less side effects.

As set forth in the specification, typical and atypical psychotropic drugs act by binding to the dopaminergic receptor (typical) or to the serotonin-2A and dopaminergic receptor (atypical). NT agonist, however, act by yet a further mechanism and do not bind to either of these receptor, yet are capable of inhibiting serotonin-2A and/or alpha-1 receptor mediated neural function. The present disclosure provides an unrecognized therapeutic drug for the treatment of various psychotic diseases and disorders that is independent of dopaminergic and serotonin-2A receptor mediated activity.

Furthermore, as the specification demonstrates DOI is an agonist of the serotonin-2A receptors not dopamine receptors. Blocking of the serotonin-2 receptor

produces therapeutic benefit for a wide variety of psychiatric disorders other than schizophrenia and even for a wider range of symptoms suffered by schizophrenia patients (negative and cognitive symptoms) than blocking of dopamine receptors alone . Blocking of alpha-1 receptors in the presence of blocking serotonin-2A receptors further enhances the ability to treat this wide range of symptoms. The specification and data show that neurotensin agonists such as NT69L and PD149163 reverse the effects of DOI and cirazoline, selective serotonin-2A and alpha-1 receptor agonists, respectively. The ability to block transmission at serotonin 2A and alpha-1 receptors were not known to be a pharmacological property of neurotensin agonists prior to the studies described in the present application.

The animal model of PPI disruption produced by drugs such as amphetamine or DOI or cirazoline provides researchers information about the pharmacological properties. A drug which reverses PPI disruption by amphetamine, a dopamine receptor agonist, may not reverse PPI disruption by DOI which is a serotonin-2 agonist and vice versa. Similarly with cirazoline, a selective alpha-1 agonist. Regardless of pathophysiology relationship, drug-induced PPI disruption provides a specific in vivo "assay" to obtain knowledge about the specific pharmacological properties of a test drug. This function of the drug induced PPI disruption rat model was a feature underlying the discovery of new, therapeutically relevant, pharmacological properties associated with neurotensin agonists such as NT69L and PD149163.

REJECTION UNDER 35 U.S.C. §103

Claims 15-16, 18, 22, 24 and 26 stand rejected under 35 U.S.C. §103 as allegedly unpatentable over Hertel *et al.* (Eur. J. Pharmacol. 422:77-81, 2001), in view of Feifel *et al.* (Brain Res. 760:80-84, 1997). Applicants respectfully traverse this rejection.

Applicant previously addressed the combination of Hertel *et al.* and Feifel *et al.* The Examiner maintains the rejection in view of Applicant's prior remarks for the following reasons.

The Office Action indicates that the mechanism of action, although argued in Applicant's prior response, was not a limitation of the claims being argued. Applicant has amended the claims (claims 15 and 16) to support the serotonin-2A mediated inhibition demonstrated by the invention (previously examined in claim 24). It is important to understand that identification of this mechanism provides for novel methods of treatment for diseases not previously recognized as being treatable with an NT agonist.

The Office Action also alleges in a post filing reference (Bennett *et al.*) that schizophrenia is an anxiety/depression disorder. Applicant respectfully disagrees with the interpretation of Bennett *et al.* particularly considering the Bennett *et al.* is a purely "speculative essay" (see, first line of "Conclusion"). Thus, Applicant respectfully submits that the reference being relied upon is a non-enabling reference with no scientific foundation for the assertion being relied upon by the Examiner.

The Office Action further alleges that hyperactivity is shown to be associated with anxiety/depression and sleep disorders (citing Svestka, Neuroendocrin Lett, 29:65-92, 2008). It is unclear to the Applicant what relevance the Svestka reference serves other than to possibly indicate hyperactivity and anxiety are sometimes associated. However, Applicant submits that each of hyperactivity and anxiety are not indicative of each other since both have their own etiology.

Furthermore, the Office Action rejections claims 24-26 because a new use for an old compound does not render the compound novel (citing to *AtlasPowder Co. v. Ireco Inc.*, 190 F.3d 1342, 1347, 51 USPQ2d 1943, 1947 (Fed. Cir. 1999). Applicant submits that the claims are not directed to a compound, but are rather directed to a method of using a compound. The Examiner will recognize that new uses for old compounds are patentable (*i.e.*, methods of use are patentably distinct from a composition). Accordingly, the citation of *AtlasPowder Co. v. Ireco Inc.* is in error because the holding is *AtlasPowder* is not relevant to the present claims.

The Office Action further alleges under this Section 103 rejection that "the claiming of a new use, new function or unknown property which is *inherently* present in the prior art does not necessarily make the claim patentable." (See Page 6 of the Office Action, emphasis ours). Applicant submits that inherency predicated upon obviousness requires far more than a mere assertion and should truly require a

teaching, suggestion or motivation (TSM) requirement. In other words, "Obviousness cannot be predicated on what is unknown." *In re Spormann*, 363 F.2d 444, 448 (C.C.P.A. 1966). Furthermore, a proper analysis for inherency requires that the inherent feature "necessarily flow" from the reference being cited. Here, it is well recognized that the etiologies of schizophrenia and bipolar, anxiety and depression are very different, thus the use of a compound for schizophrenia does not necessarily suggest and effective therapy for bipolar disease and disorders, anxiety diseases and disorders or depression diseases and disorders. This is evidenced by the use of different drugs to treat schizophrenia vs., for example, bipolar diseases. In other words, use of one drug to treat schizophrenia *does not necessarily* mean the drug can be used to treat depression.

Thus, Hertel *et al.* in combination with Feifel *et al.* fail to teach or suggest (i) improving symptoms by increasing sensorimotor gating in a subject having a bipolar disease or disorder, an anxiety disease or disorder or a depression disease or disorder (see, *e.g.*, claims 15 and 16); and (ii) fail to teach or suggest any aspect of claim 24.

Hertel *et al.* and Feifel *et al.* teach that NT agonists reverse amphetamine-induced increase in locomotor activity and amphetamine-induced prepulse inhibition, respectively. As described above, amphetamine produces these effects by stimulation of the dopamine receptor function. Hertel *et al.* and Feifel *et al.* thus teach and suggest that NT69L blocks stimulation at dopamine receptors. However, in contrast, the disclosure demonstrates NT agonist including NT69L and PD149163 block stimulation of 5-HT2A receptors since it blocks PPI disruption by DOI which reduces PPI by activation of 5-HT2A, but not dopamine, receptors.

Nothing in Hertel *et al.* or Feifel *et al.* teach or suggest the use of an NT agonist in the treatment of bipolar diseases or disorders, anxiety diseases or disorders or depression disease or disorders. Furthermore, the references when combined do not teach or suggest modulating any of the disease or disorders above as described in claim 24. Nothing in the references teach or suggest a role of NT agonist as putative atypical antipsychotics that modulate serotonin and dopaminergic pathways without interacting with the respective receptors (5HT-2A and D2, respectively). Because Hertel *et al.* and Feifel *et al.* recognized only the ability to

modulate dopaminergic receptors a biochemical effect that, by itself, has only a limited ability to improve symptoms of psychiatric disorders, namely positive symptoms in schizophrenia, but not the symptom spectrum in other disorders such as bipolar disorder, depression and anxiety. In contrast, modulation of serotonin receptors, by itself or in combination with modulation of dopamine receptors has been demonstrated to produce improvements in a broad spectrum of psychiatric disorders.

In contrast to Hertel *et al.* and Feifel *et al.*, the disclosure demonstrates that NT agonist have a broader spectrum of biochemical effects than just inhibition of dopamine receptors, namely that NT agonists modulate serotonin-2A receptors. This discovery demonstrates that NT agonists have a potential to treat a wider range of psychiatric symptoms that previously assumed when they were know only to modulate dopamine receptors.

Claims 15-17 and 25 stand rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Hertel *et al.* and Feifel *et al.* in view of Costa *et al.* (Eur. J. Pharm. 428:97-103,2001). Applicant respectfully traverses this rejection.

Applicant incorporates the comments above with respect to this rejection. Costa *et al.* is combined with Hertel *et al.* and Feifel *et al.* to allegedly provide the element of treating a subject with SR48692 and SR142948. Applicant submits that the piecing together of elements of the disclosure fails to view the invention as a whole.

Taken as a whole, the disclosure demonstrates that NT agonist provide a class of novel drugs useful for a broader spectrum of diseases and symptoms disorder that could not otherwise be treated with therapeutics that modulate dopaminergic receptor activation alone (as allegedly taught by the cited references.). Taken as a whole, the combination of Hertel *et al.*, Feifel *et al.* and Costa *et al.* fail to teach or suggest the treatment of bipolar diseases or disorders, anxiety diseases or disorders or depression disease or disorders.

At the root of a prima facie obviousness rejection is the fact that the elements set forth in the claims must be taught by the reference or references when combined. The combination of Hertel *et al.*, Feifel *et al.* and Costa *et al.* do not teach or suggest

each and every element of the claimed invention and thus fails to provide a prima facie case of obviousness.

For, at least, the foregoing reasons the claims submitted herewith are non-obvious over the references either alone or in combination.

For at least the foregoing, the Applicant submits that the claimed invention is patentable and request reconsideration and notice of such allowable subject matter.

The Director is authorized to charge any required fee or credit any overpayment to Deposit Account Number 50-4586, please reference the attorney docket number above.

The Examiner is invited to contact the undersigned at the below-listed telephone number, if it is believed that prosecution of this application may be assisted thereby.

Respectfully submitted,

GAVRILOVICH, DODD & LINDSEY LLP

Date: December 4, 2009

By: /Joseph R. Baker, Jr./
Joseph R. Baker, Jr.
Registration No. 40,900

4660 La Jolla Village Drive, Suite 750
San Diego, California 92122
(858) 458-3607 (Main)
(858) 458-9986 (Fax)

APPENDIX A

Critical Reviews in Toxicology, 38:489–542, 2008

ISSN: 1040-8444 print / 1547-6898 online
DOI: 10.1080/10408440802014238

informa
healthcare

An Assessment of Anxiolytic Drug Screening Tests: Hormetic Dose Responses Predominate

Edward J. Calabrese
Department of Public Health, School of Public Health and Health Sciences, Environmental Health Sciences Division, University of Massachusetts, Amherst, Massachusetts, USA

This article provides a comprehensive assessment of dose response relationships for anxiolytic (i.e., anxiety-reducing) agents within a broad representation of the animal model screening tests. These screening tests include the elevated plus maze test, light–dark test, hole board test, open field test, four plates test, social interaction test, Vogel's conflict test, staircase test, freeze behavior test, forced swimming test, tail suspension test, communication box test, and immobilization/cold stress test. The analysis revealed that hormetic-like biphasic dose responses were commonly observed across all screening tests, independent of the animal model, the conditions of the test, modifications of tests by investigators, and the chemical class of agents tested. The quantitative features of the dose response, as measured by the magnitude and width of the stimulation at low doses, were similar across all screening tests and experimental conditions, regardless of the mechanism and receptor activation pathway identified. These findings, which add more support to the perspective that the hormetic dose response represents the most fundamental and common dose-response model in the biomedical and toxicological sciences, have important implications for the process of drug discovery/development, clinical evaluation, and quantitative expectation of drug treatment effects.

Keywords anxiety, anxiolytic, biphasic, conflict test, depression, dose-response, elevated plus maze test, forced swimming test, four plates test, GABA, hole board test, hormesis, light–dark test, open field test, partial agonist, social interaction test, staircase test, stress, tail suspension test, ulcer, U-shaped

Experimental behavioral pharmacology is strongly driven by the pharmaceutical industry and governmental research funding initiatives, typically leading to the development of new and improved drugs or drug applications with the intention of improving human lives in a variety of ways. As a group, anti-anxiety drugs have received extensive evaluation in the biomedical literature, with particular emphasis on a broad spectrum of anxiolytic drug screening tests (File and Hyde, 1978; File, 1980; Treit, 1985; Hogg, 1996; Kulkarni and Reddy, 1996; Bourin, 1997; Hascoet et al., 2001; Borsini et al., 2002; Sanchez, 2003; Prut and Belzung, 2003; O'Neil and Moore, 2003; Bourin and Hascoet, 2003; Ripoll et al., 2003; McArthur and Borsini, 2006). While such screening tests can serve a wide range of research interests, the frequent inclusion of a large number of doses often permits a detailed evaluation of the dose-response relationship. These powerful study designs have often been coupled or sequenced with the use of synthetic agonists and/or antagonists that have led to the identification of mechanistic pathways by which the dose response is affected. Despite the robustness of the experimental data bases for anxiolytic drug screening, a detailed and integrated analysis of general dose response features of this class of drugs has been surprisingly lacking. Nonetheless, the dose-response concept is of fundamental importance in the process of drug discovery, development, and clinical evaluation, being able to significantly impact potential success at each step.

The present analysis provides a detailed assessment of the dose-response features and the underlying mechanistic framework for the broad body of anxiolytic drugs based on the most commonly employed and validated screening tests. Of particular significance is that the most commonly observed dose-response relationship is the inverted U-shape, which displays the same quantitative features of the hormetic dose-response relationship, a model widely reported in other biomedical sciences, where responses are independent of biological model, endpoint, and chemical class. While these findings have general significance concerning the fundamental nature of the dose response, they

Address correspondence to Edward J. Calabrese, PhD, Professor of Toxicology, Department of Public Health, School of Public Health and Health Sciences, Environmental Health Sciences, Morrill I, N344, University of Massachusetts, Amherst, MA 01003, USA. E-mail: edwardc@schoolph.umass.edu

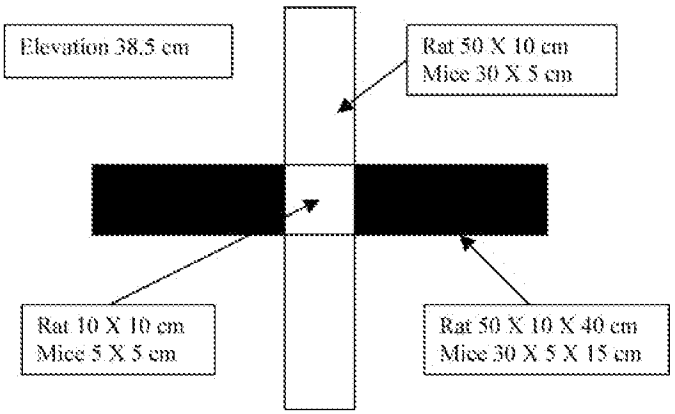

FIG. 1. Aerial view of elevated plus mazes for mice and rats. Dimensions of each apparatus are indicated. (Source: Onaivi et al., 1990).

may also have particular relevance to drug discovery in general and anxiolytic drugs in particular, leading to strengthened study designs in animal screening tests and in clinical trials, and reducing the risk of missing candidate drugs.

This article provides a detailed assessment of the most significant anxiolytic screening tests, with each specific test section containing an initial brief description of the screening test, followed by a detailed evaluation of the dose response, including its qualitative and quantitative features and mechanistic basis. Particular emphasis is given in each screening test section for the inclusion of graphs of representative hormetic-like dose responses. A concluding discussion section is included that integrates the findings of the individual screening tests and the biomedical implications for the hormetic dose response in the assessment of anxiolytic drugs.

ELEVATED PLUS MAZE (EPM) TEST

The elevated plus maze (EPM) test is commonly employed as an initial screening evaluation for the assessment of potential anxiolytic agents. It is an experimental system that is easy to use and yields data in an efficient manner. The EPM test has the advantage of needing no prior training of animals, nor the use of food or water deprivation. Despite these important advantages, the EPM test has limitations since the animals cannot be reused due to possible habituation. Even though this test has broad predictability, there are some agents or classes of agents, such as 5-HT_{1A} agonists, that may yield false negatives.

The EPM test offers a procedure that takes advantage of the natural exploratory behavior of rodents once they are placed in an elevated maze, consisting of two (opposite) open and two (opposite) closed (walled) alleys (Figure 1). The animals explore the different alleys, with the open-arm alleys being more aversive than the closed ones. Anxiolytic drugs aid in overcoming the fear-induced inhibition of open alley exploration. The typical endpoints measured include the number of open and closed alley entries and the time spent in each alley. Occasionally, other endpoints are measured, including frequency and duration of "scanning," which is the protruding of the head over the edge of an open arm and fanning with the vibrissae (i.e., hair growth at the nares) in any direction, "risk assessment," which is the protruding from an enclosed arm with the forepaws and head only, and "end activity," which is the amount of time spent at the end of an open arm.

The EPM test was developed by Pellow et al. (1985) and Pellow and File (1986) to assess anxiolytic and anxiogenic drug effects on exploratory activity in rodents. This 1986 paper became widely accepted and has been cited in about 1,700 papers in the Web of Science database some 20 years later.

There can be considerable variation in experimental protocols when the EPM is used. For example, in some studies the animal is placed facing an open alley while in other experiments it may be placed facing a closed alley. How behavior is scored can also differ. Typically four paws are required to be placed in the alley for a "score," but some times only two paws have been required. The duration of observation may also vary from 3 to 5 min. The 5-min period was initially selected because Montgomery (1958) showed that the avoidance behavior was quite marked during this period; however, it began to decrease by the end of a 10-min period. Of significance is that performance in the EPM was consistent when repeated over multiple days, not making it susceptible to the so-called "one-day-tolerance" phenomenon as seen in the Four Plates Test. These factors have contributed to making the EPM widely used since it can be adapted to the individual needs of investigators while offering the capacity for reproducible findings within a screening protocol where the endpoints are interpretable within the context of the biological/psychological understandings of anxiety.

A wide range of compounds has displayed biphasic dose responses in the EPM (Figure 2, a–t). As seen in Table 1, these diverse agents likely act via a broad array of

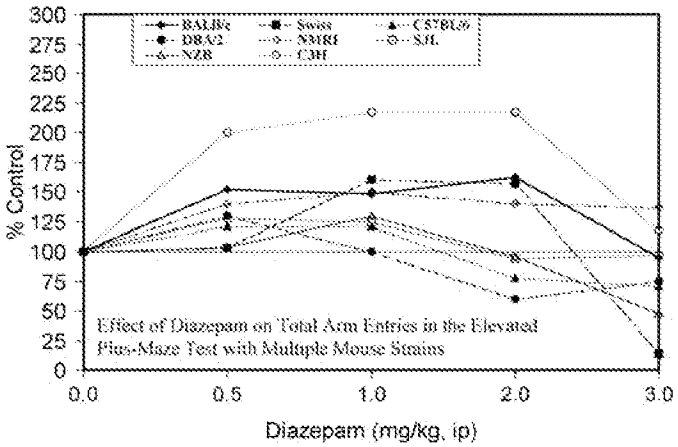

FIG. 2a. Effects of diazepam on total arm entries in the elevated plus-maze test with multiple mouse strains. (Source: Griebel et al., 2000a, Figure 4, p. 167).

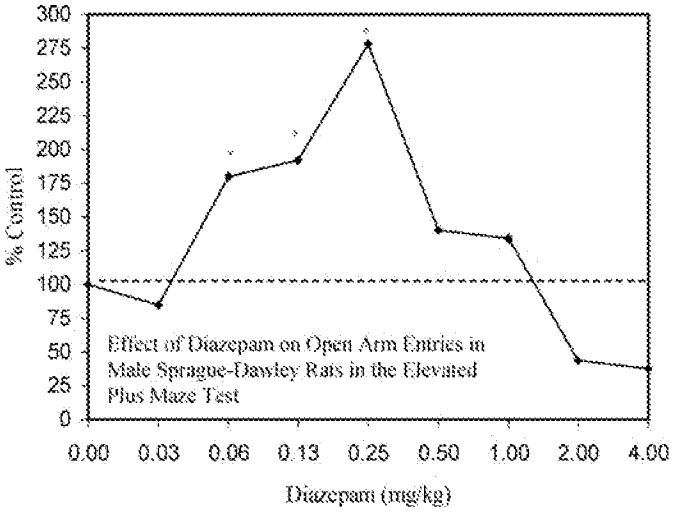

FIG. 2b. The effect of diazepam on open arm entries in male Sprague-Dawley rats in the elevated plus-maze test. *Significantly different from controls at $p < .05$. (Source: Moser et al., 1990, Figure 5, p. 347).

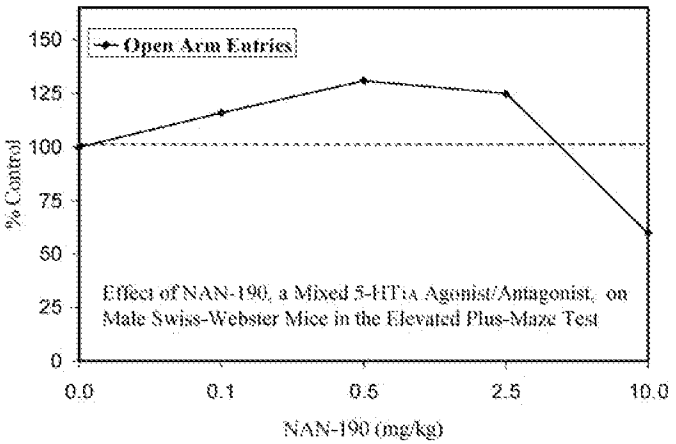

FIG. 2d. Effect of NAN-190, a mixed 5-HT_{1A} agonist/antagonist, on male Swiss-Webster mice in the elevated plus-maze test. (Source: Cao and Rodgers, 1997, Table 1, p. 599).

pharmacological receptors, including the gamma-aminobutyric acid (GABA)/benzodiazepines (Figure 2, a and b), the 5-hydroxytryptamine (5-HT) family (i.e., 5-HT_{1A}, 5-HT_3, and 5-HT_4) (Figure 2, c–f), adrenergic (Figure 2g), cholecystokinin (CCK) (Figure 2h), dehydroepiandrosterone (DHEA) (i.e., GABA receptor) (Figure 2i), dopamine (Figure 2j), NK (Figure 2, k and l), nitrous oxide (i.e., benzodiazepine receptor) (Figure 2, m and n), *N*-methyl-D-aspartate (NMDA) (Figure 2o), and adenosine (Figure 2p), among others (Figure 2, q–t). An analysis of the dose responses of numerous agents acting through about a dozen different receptors reveals strikingly similar quantitative features of the dose response as seen by the magnitude and width of the stimulatory responses. The biphasic responses in these experimental conditions have been assumed to result from a mixed agonist/ antagonist effect (Melchior and Ritzmann, 1994a, 1994b; Momose et al., 1998) in which the agent acts at two sites, a high-affinity stimulatory site and a low-affinity inhibitory site (Smith et al., 2004).

The magnitude of the stimulatory response is typically less than twice that of the control group, being usually only 30–60%

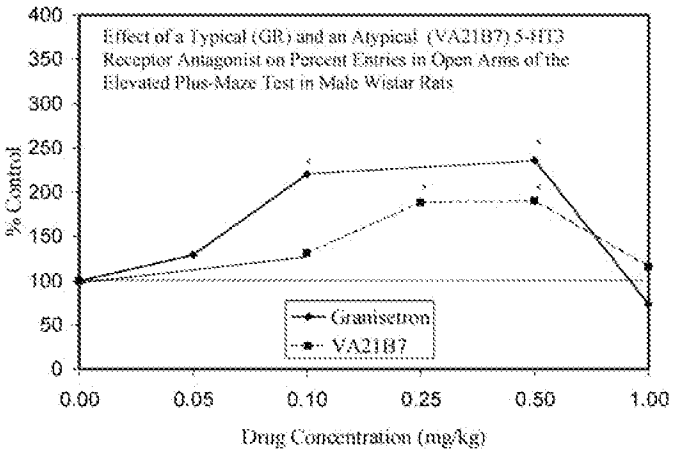

FIG. 2c. Effect of a typical (granisetron) and an atypical (VA21B7) 5-HT_3 receptor antagonist on percent entries in open arms of the elevated plus-maze test in male Wistar rats. *Significantly different from controls at $p < .05$. (Source: Artaiz et al., 1995, Table 1, p. 143).

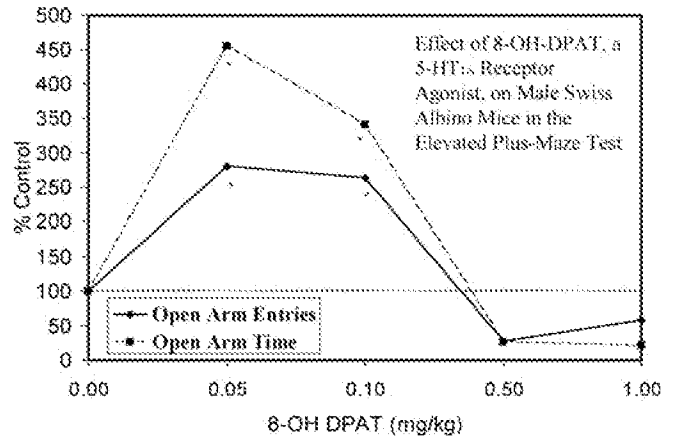

FIG. 2e. Effect of 8-OH-DPAT, a 5-HT_{1A} receptor agonist, on male Swiss Albino mice in the elevated plus-maze test. *Significantly different from controls at $p < .05$. (Source: Nunes de-Souza et al., 2000, Table 1, p. 303).

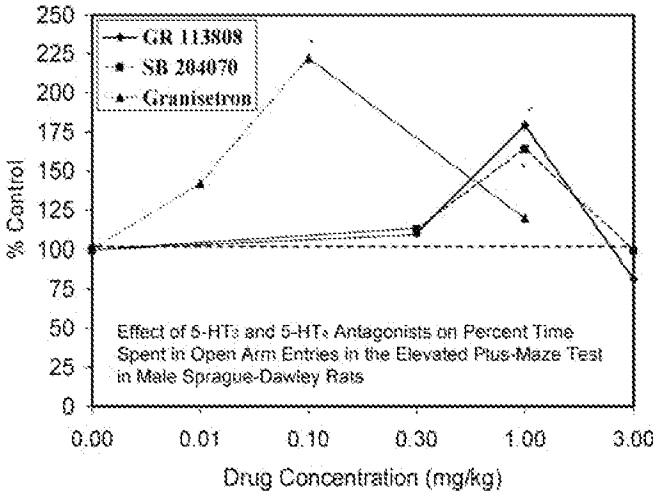

FIG. 2f. Effects of 5-HT_3 (granisetron) or 5-HT_4 (GR 113808 and SB 204070) antagonists in the elevated plus maze test in male Sprague-Dawley rats. *Significantly different from controls at $p < .05$. (Source: Silvestre et al., 1996, Table 1, p. 221).

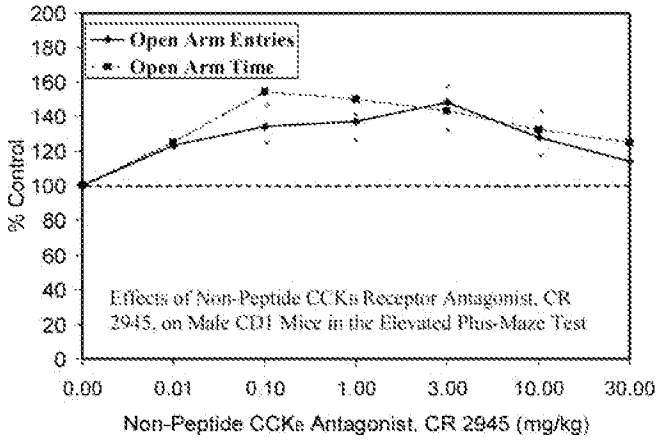

FIG. 2h. Effects of the non-peptide CCK_B receptor antagonist, CR 2945, on male CD1 mice in the elevated plus-maze test. *Significantly different from controls at $p < .05$. (Source: Revel et al., 1998, Figure 1, p. 188).

greater than control at maximum, although there are exceptions to this general perspective. The width of the stimulatory response was usually within 20-fold dose range of the estimated threshold, although there were several cases when the stimulatory range approached and exceeded 1,000-fold (e.g., CR 2945, Figure 2h; DHEA, Figure 2i; motilin, Figure 2r; and cannabidiol, Figure 2s). These quantitative features of the dose response are generally similar not only across agents and receptors but also across various strains of mice, rats, and other models such as gerbils. These findings indicate that there is a performance (i.e., stimulatory magnitude) limitation with respect to a drug-induced anxiolytic (and anxiogenic) effect consistent with the pharmacological ceiling effect. Regardless of the receptor-based initiating pathway, the dose-response features are similar, suggesting biological plasticity within which the response is constrained. Even manipulation of the binding sites does not necessarily alter these quantitative features of the dose response. For example, the anxiolytic effect induced by low doses of diazepam is associated with allosteric benzodiazepine sites. Deletion of the type I GABA subunit binding site in knockout mice effected a compensatory increase in Type II GABA binding sites, which modulated the magnitude of the dose response but still resulted in behavioral responses consistent with the

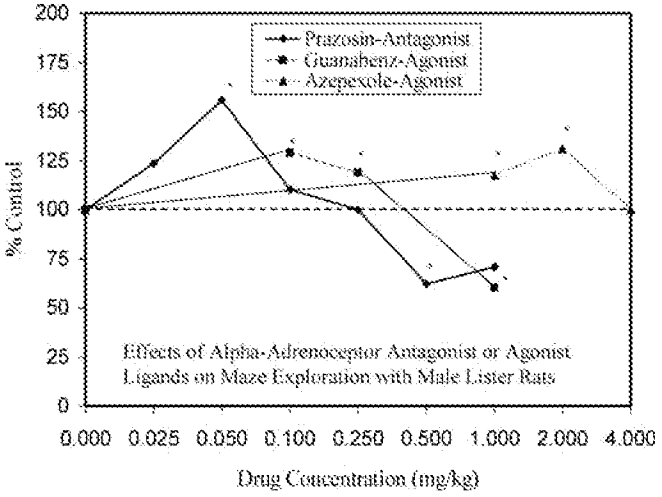

FIG. 2g. Effects of α-adrenoceptor antagonist or agonist ligands on maze exploration with male Lister rats. *Significantly different from controls at $p < .05$. (Source: Handley and Mithani, 1984, Tables 2 and 3, p. 3).

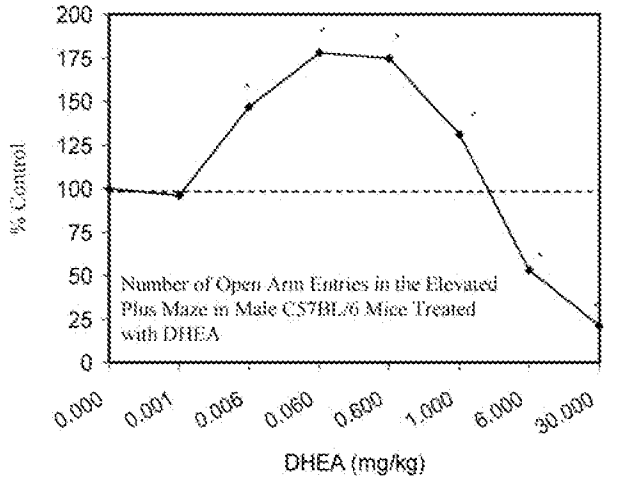

FIG. 2i. Number of open arm entries in the elevated plus maze in male C57BL/6 mice treated with DHEA. *Significantly different from controls at $p < .05$. (Source: Melchior and Ritzmann, 1994b, Figure 1, p. 439).

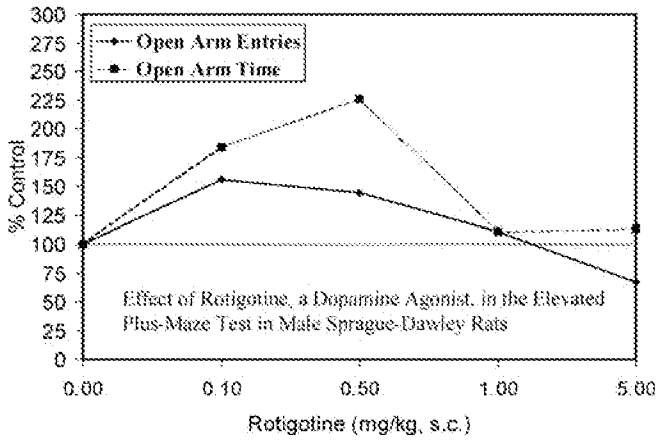

FIG. 2j. Effect of rotigotine, a dopamine agonist, in the elevated plus-maze test in male Sprague-Dawley rats. (Source: Bertaina-Anglade et al., 2006, Table 1, p. 110).

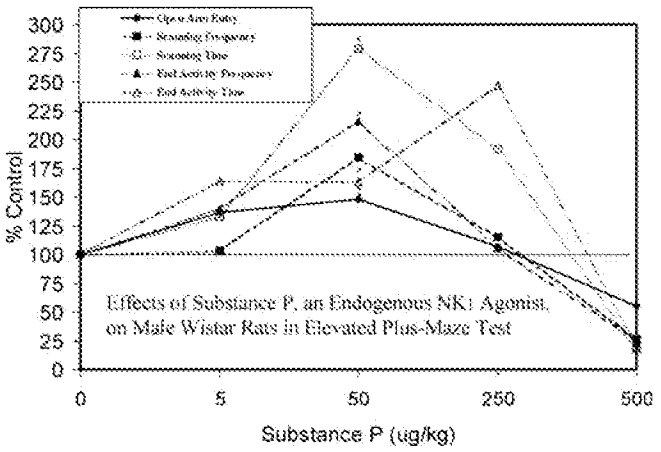

FIG. 2l. Effects of substance P, an endogenous NK_1 agonist, on male Wistar rats in elevated plus-maze test. *Significantly different from controls at $p < .05$. (Source: Hasenohrl et al., 1998, Table 1, p. 127).

quantitative features of the hormetic dose response (Kralic et al., 2002).

Summary of Several General Research Areas

(1) NMDA Antagonists and Anxiolytic Effects

In the early 1990s anxiolytic-like effects were reported for the NMDA receptor antagonist 10-α-amino-7-phosphonoheptanoic acid (AP7) following injection into the dorsal periqueductal grey (DPAG) region of male Wistar rats using the elevated plus maze (Guimaraes et al., 1991). These findings confirmed and extended earlier studies of Stephens et al. (1986) demonstrating that NMDA antagonists induced anxiolytic-like effects when administered peripherally. These two key observations led Guimaraes et al. (1991) to hypothesize that nitric oxide, which activates NMDA receptors in the DPAG, may increase anxiety. Several follow-up studies have been based upon this association between NO and NMDA receptors. Using the elevated plus maze, L-NOARG and L-NAME, two inhibitors of nitric oxide synthase (NOS) (Guimaraes et al., 1994), and methylene blue (MB), a vital nontoxic dye (de Oliveira and Guimaraes, 1999; Eroglu and Caglayan, 1997) with notable effects on the nitrergic system, including its inhibition of NOS (Mayer et al., 1993), were assessed with male Wistar rats. In the case of MB, it was either injected into the tail vein (Eroglu and Caglayan, 1997) or microinjected into the DPAG (de Oliveira and Guimaraes,

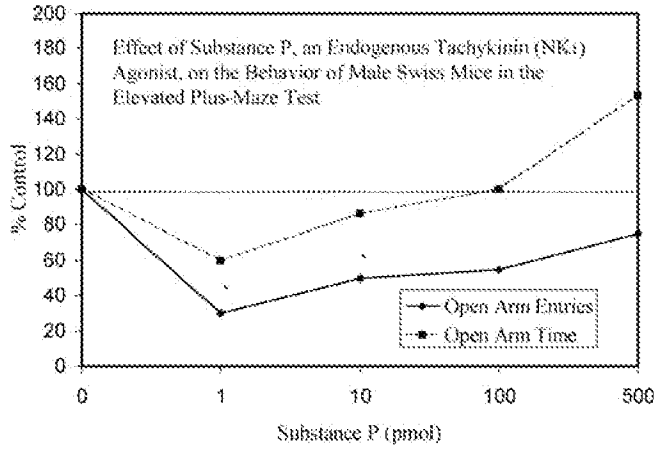

FIG. 2k. Effects of substance P, an endogenous NK_1 agonist, on the behavior of male Swiss mice in the elevated plus-maze test. *Significantly different from controls at $p < .05$. (Source: Teixeira et al., 1996, Figure 1A, p. 9). Note that this agent is inducing an anxiogenic response at low doses.

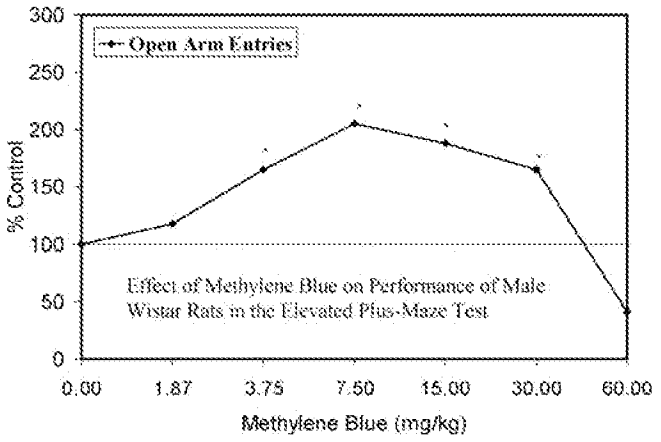

FIG. 2m. Effect of methylene blue on performance of male Wistar rats in the elevated plus-maze test. *Significantly different from controls at $p < .05$. (Source: Eroglu and Caglayan, 1997, Figure 1, p. 383).

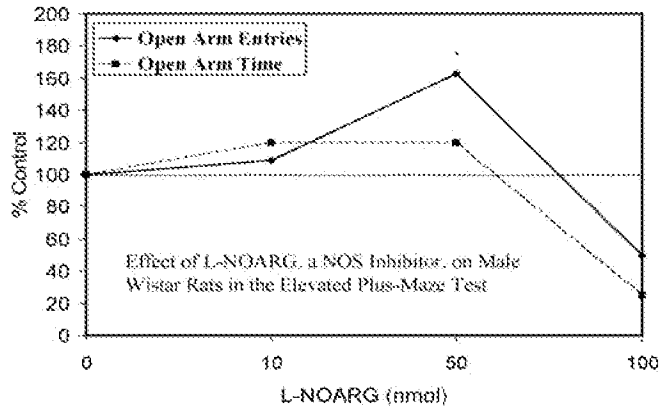

FIG. 2n. Effect of L-NOARG, a NOS inhibitor, on male Wistar rats in the elevated plus-maze test. *Significantly different from controls at $p < .05$. (Source: Guimaraes et al., 1994, Figure 3, p. 1931).

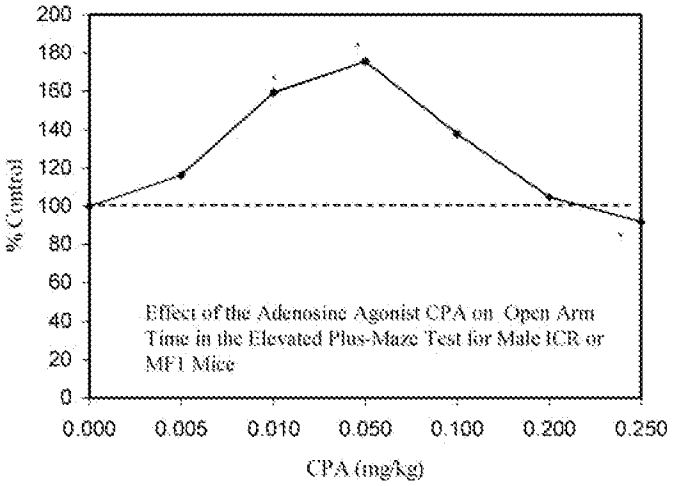

FIG. 2p. Percentage of open arm time in the elevated plus-maze test for male ICR or MF1 mice treated with the adenosine agonist CPA. *Significantly different from controls at $p < .05$. (Source: Jain et al., 1995, Figure 2, p. 2128).

1999) as was done for L-NAME and L-NOARG (Guimaraes et al., 1991). Regardless of the respective study designs, which used from three to six doses, the results produced U-shaped dose responses (Figure 2, n and o). The quantitative features of the dose response in each case were consistent with the hormetic-like biphasic dose response with respect to the maximum stimulatory response and the width of the stimulatory range. Eroglu and Caglayan (1997) speculated that NO has either an enhancing or inhibitory role in the generation of anxiety, depending on the magnitude of NOS inhibition.

The MB treatment not only was anxiolytic but also was an antidepressant, based on the forced swimming test. This antidepressant effect likewise displayed the U-shaped dose response. Despite the presumption that MB-induced anxiolytic and antidepressant effects appear to be related to its inhibition of NOS and production of endogenous NO, the optimal dose for the anxiolytic response occurred at a lower dose (7.5 vs. 15.0 mg/kg) than for the antidepressant effects.

(2) Tachykinin Receptors (TKR)

The tachykinin receptor (TKR) family was shown by Teixeira et al. (1996) to modulate anxiety responses in the EPM test in male Swiss mice via central mechanisms that were assessed by intra cerebroventricular administration of various TKR agonists and antagonists. Both NK_1 (e.g., Substance P) (Figure 2k) and NK_2 agonists induced a J-shaped dose response in the EPM based on entries and time spent in open arms of the maze.

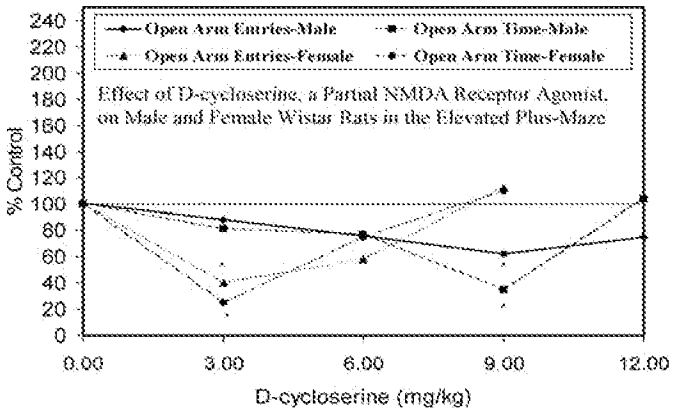

FIG. 2o. Effect of D-cycloserine, a partial NMDA receptor agonist, on male and female Wistar rats in the elevated plus-maze. *Significantly different from controls at $p < .05$. (Source: Ferreira and Morato, 1997, Figure 2, p. 1640). Note that this drug is inducing an anxiogenic response at low doses.

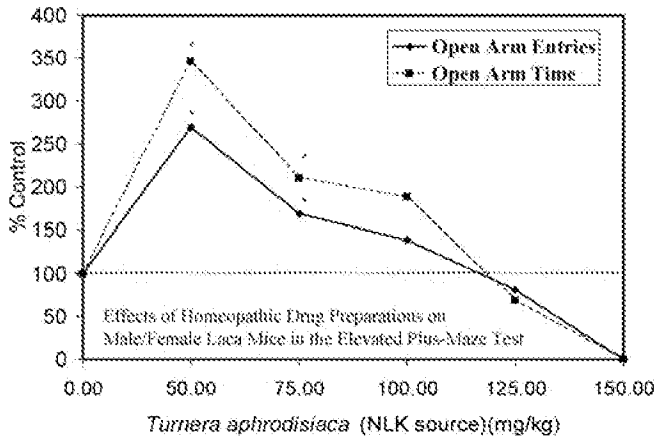

FIG. 2q. Effects of homeopathic drug preparations (dried moth tinctures of *T. aphrodisiaca*) in Laca mice in the elevated plus-maze test. Significantly different from controls at $p < .05$. (Source: Kumar and Sharma, 2005, Table 1, p. 119).

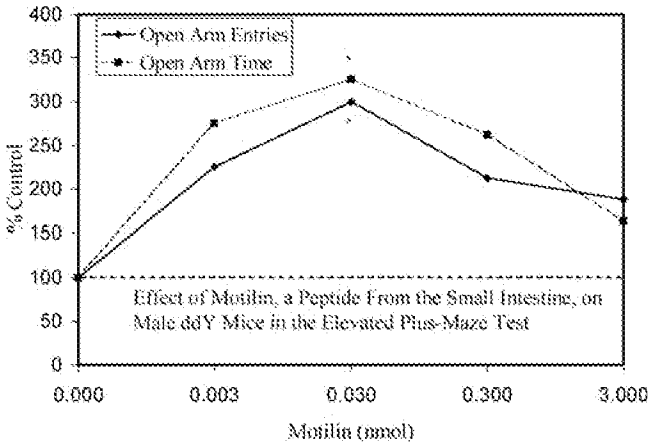

FIG. 2r. Effect of motilin, a peptide from the small intestine, on male ddY mice in the elevated plus-maze test. *Significantly different from controls at $p < .01$. (Source: Momose et al., 1998, Figure 1, p.1740).

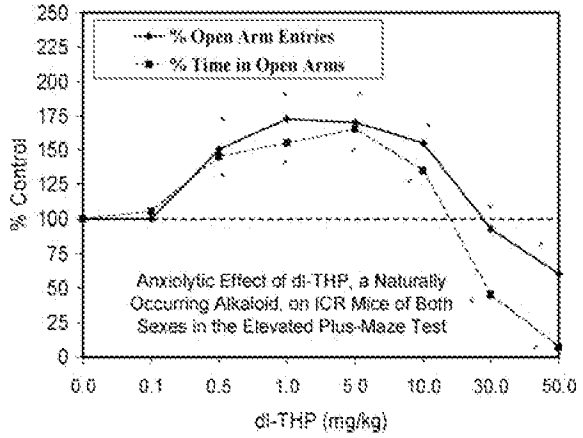

FIG. 2t. Anxiolytic effect of dl-THP, a naturally occurring alkaloid, on ICR mice of both sexes in the elevated plus-maze test. *Significantly different from controls at $p < .05$. (Source: Leung et al., 2003, Figure 2, p. 777).

That is, low doses of these agonists increased anxiety while higher doses reversed this effect. However, the administration of synthetic antagonists for these respective TKRs displayed inverted U-shaped dose responses, with anxiety being reduced at low doses (Teixeira et al., 1996). NK_1 antagonists were also anxiolytic in gerbils with the EPM test (Varty et al., 2002a, 2002b). It is interesting to note that Substance P, which was shown to induce a J-shape dose response in male Swiss mice in the EPM, induced a marked inverted U-shape in the same test in male Wistar rats (compare Figure 2, k and l). That is, in the male Swiss mouse model Substance P was anxiogenic at low doses, whereas the opposite was the case in the male Wistar rat model.

These findings are particularly interesting since the modulation of anxiety responses by the NKR agonists and antagonists did not affect locomotor activity and motor performance in the rotarod test, thereby decoupling the anxiety response from these endpoints. Such findings are notable since they provide a means of separating anxiety from sedation, a factor that can obscure the actual dose response since it may affect performance.

Even though this study used synthetic antagonists, the findings suggest the possible existence of endogenous antagonists of TKR receptors that may serve a modulatory function. However, these findings reveal that $NK_1$and NK_2 endogenous agonists biphasically affected the anxiety response and may themselves be part of a broader modulatory scheme. Regardless of whether the responses were of a J- or inverted U form, the TKR agonists and antagonists displayed dose-response relationships that were fully consistent with the hormetic dose response with respect to the magnitude and width of the stimulatory response.

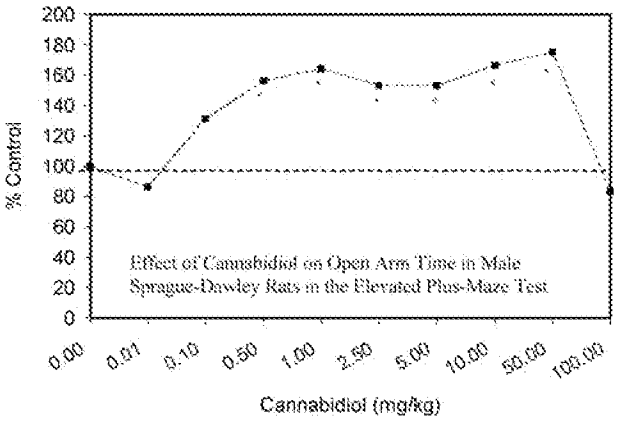

FIG. 2s. Effects of cannabidiol on male Sprague-Dawley rats in the elevated plus-maze test. *Significantly different from controls at $p < .05$. (Source: Onaivi et al., 1990, Table 1, p. 1006).

(3) Seizure-Inducing Agent (PTZ): Anxiolytic at Low Doses

The seizure-inducing model agent pentylenetetrazol (PTZ) was assessed in the EPM at doses far below its capacity to cause seizures even at the highest dose tested. Using adult male DBA/2 mice, PTZ affected an hormetic-like biphasic dose response, with anxiolytic effects occurring at low and anxiogenic effects at higher doses (Rodgers et al., 1995). This was demonstrated by an enhancement of open entries and shorter entry latency at low doses, while the opposite occurred at higher doses (Figure 3). The mechanism by which PTZ affects anxiety-related behavior is most likely due to its capacity to act via the picrotoxin site on the benzodiazepine/GABA receptor complex to alter the chloride influx (Pellow and File, 1986).

(4) 5-HT

Drugs stimulating the $5\text{-}HT_{1A}$ receptor have been broadly viewed as not displaying anxiolytic effects while often causing

TABLE 1
Agents inducing biphasic dose responses in EPM

Motilin
Acts via motilin receptor
Substance P/Substance P Methyl Ester
Acts via a NK_1 receptor
Rotigotine
Acts via a dopamine agonist
Propericiazine
Acts via a dopamine receptor
Methylene Blue
Acts via the nitric oxide pathway
L-NAME
Acts via the nitric oxide pathway
L-NOARG
Acts via the nitric oxide pathway
Cannibinoid
Acts via a benzodiazepine receptor
Pregnenolone Sulfate
Acts via $GABA_A$ receptor
Paeonol
Acts via a MAO receptor
Buspirone
Acts via 5-HT_{1A} and 5-HT_{3A} receptors
Way-102635
Acts via 5-HT_{1A} and 5-HT_{3A} receptors
NAN-190
Acts via 5-HT_{1A} and 5-HT_{3A} receptors
8-OH-DPAT
Acts via 5-HT_{1A} and 5-HT_{3A} receptors
Alcohol
Acts via the $GABA_A$ receptor
Naloxone
Acts via the opiate receptor
CR2945/CR1795
Acts via the CCK receptor
MCI-225
Acts via an adrenergic receptor
Dehydroepiandrosterone (DHEA) and its sulfate (DHEAS)
Acts via the GABA receptor
Neurokinin A
A NK_2 receptor agonist
FK888
A NK_1 antagonist
SR 48968
A NK_2 antagonist
dl-Tetrahydropalmitine
Acts at $GABA_A$ receptor benzodiazepine site
Diazepam
Acts via 5-HT_{1A} receptor
MDL 7005EF
Acts via 5-HT_{1A} receptor

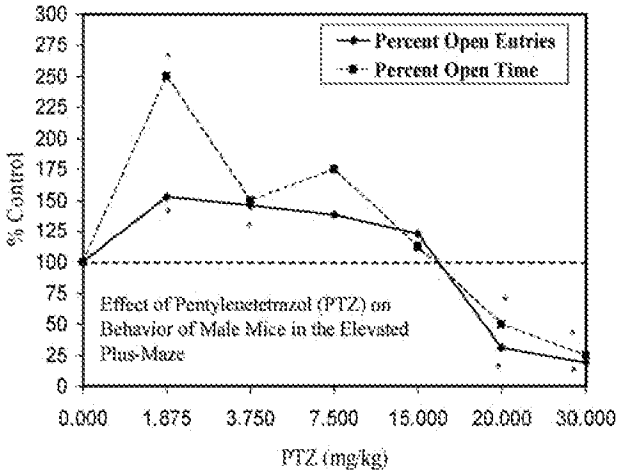

FIG. 3. Effects of pentylenetetrazol (PTZ) on the behavior (percent open entries; percent time spent in open part of the maze) of male mice in the elevated plus-maze. *Significantly different from controls at $p < .05$. (Source: Rodgers et al., 1995, Figure 3, p. 809).

the opposite, that is, being anxiogenic (File et al., 1987; Pellow et al., 1985; Critchley and Handley, 1987; Moser, 1989; Griebel, 1995). However, a variety of experimental interventions (Hjorth et al., 1987; Kahn et al., 1988; Soubrie, 1986) that result in decreased and increased brain serotonergic activity may induce anxiolytic and anxiogenic-like actions, respectively (Soderpalm et al., 1989).

This dose-response ambiguity was unintentionally clarified in research that was designed to assess possible anxiolytic-like effects of 5-HT active drugs without the confounding of either consummatory behavior or punishment, which are often invoked in behavioral studies with 5-HT. In this study with male Sprague-Dawley rats, Soderholm et al. (1989) reported that a variety of 5-HT_{1A} receptor agonists (i.e., buspirone, gepirone, ipsapirone, 8-OH-DPAT) and L-5-HTP (L-5-hydroxytryptophan, a 5-HT precursor) displayed hormetic-like biphasic dose responses in Montgomery's conflict test, which is the EPM test. The doses used in this study were generally lower than reported in the earlier cited papers and may account for their lack of ability to detect the anxiolytic response.

It was suggested that the biphasic dose response may result from a metabolic balancing function between activation of pre- and postsynaptic 5-HT_{1A} receptors in anxiety-related neuronal pathways. The dose-response shift may result from a greater sensitivity of the pre–synaptic (auto) receptor as compared to postsynaptic 5-HT_{1A} receptors, thus accounting for the narrow anxiolytic dose window of these 5-HT_{1A} agonists.

(5) Adenosine Agonists

That adenosine may affect anxiety behavior has been suggested by numerous studies indicating that the adenosine

antagonist caffeine can induce anxiogenic-like activity in animal models and humans (Baldwin et al., 1989; Charney et al., 1985; Loke et al., 1985; Uhde et al., 1984; Imaizumi et al., 1994). In addition to these suggestive caffeine studies implicating a role of adenosine in anxiety behavior, adenosine is well known for its capacity to modulate the central neuron system activity pre- and postsynaptically. Presynaptic activation of A_1 adenosine receptors typically inhibits the release of neurotransmitters including glutamate, acetylcholine, dopamine, 5-HT, and noradrenaline (Stone and Simmonds, 1991). In contrast, the A_2 adenosine receptors increase the release of these endogenous agonists. On the postsynaptic side, adenosine can increase potassium and chloride conductance while affecting neuronal sensitivity to acetylcholine and dopamine.

Based on such experimental foundations, Jain et al. (1995) assessed the effects of cyclopentyladenosine (CPA), a selective A_1 receptor agonist, on anxiety-like behavior using the elevated plus maze test with adult male ICR or MF1 mice. Testing was done 30 min after ip administration of the drug using routine evaluation procedures with a built-in replication and a study design that included 6 treatment levels over a 50-fold (0.005–0.25 mg/kg) dose range. The authors reported an hormetic-like biphasic dose response with a maximum increase in open arm entries of about 175% as compared to the control (100%) (Figure 2p). The stimulatory dose range was in the approximate range of 10-fold. However, the A_1 antagonist CPX completely blocked the anxiolytic effect of CPA.

While these findings closely linked the anxiolytic behavior with A_1 receptor activation, exploration of A_2 receptor involvement added considerable complexity to developing biologically-based mechanistic understandings. Since the A_2 agonist DPMA had no impact on anxiolytic behavior in the EPM test by itself, these initial findings seem to clearly show A_1 but no A_2 involvement in affecting the anxiolytic response. However, for some unexplained reason, the investigators tested both A_1 and A_2 agonists at the same time. In this case, the A_2 agonist inhibited the A_1receptor-induced anxiolytic effect of the CPA. A comparable inhibition by A_2 receptors has been reported by Ferre et al. (1991) with dopamine 2 receptors. Similarly, A_2 receptor suppression of A_1 receptor activation was proposed to account for the observations that low, but not high, doses of adenosine receptor agonists suppress amino acid release (Simpson et al., 1992).

Interaction studies with anxiogenic doses of caffeine and subanxiolytic or anxiolytic doses of CPA were subsequently conducted. Subanxiolytic doses of CPA completely prevented the caffeine-induced anxiogenic effect. Similarly, an anxiogenic dose of caffeine prevented the anxiolytic effect of CPA. These findings suggest that the anxiogenic-like effects of caffeine may be due to its simultaneous blockage of A_1 and A_2 receptors, for which caffeine has similar binding affinities.

Adenosine A_1 receptors have also been implicated in reducing anxiety behavior in mice during active ethanol withdrawal (Concas et al., 1994; Kaplan et al., 1999; Prediger et al., 2004), using a variety of standard behavior tests, such as the open field test and the EPM test. Of particular practical interest is that adenosine agonists may offer important advantages over treatments with benzodiazepines, based on fewer concerns with side effects.

In retrospect, it has been learned that anxiolytic effects in standard behavioral tests have been mediated in some mouse models by benzodiazepine and adenosine receptors. That is, quite independently of each other, anxiolytic effects can be induced by adenosine A_1 receptor agonists and BDZ agonists. Both effects have been blocked by receptor antagonists. Both receptor initiated events have the same dose-response features. In light of findings that an A_2 agonist blocked A_1 agonist activation of anxiolytic effects, it would be of interest to assess the effects of two anxiolytic drugs administered simultaneously as well as an A_1 agonist and BZ antagonists (and their reverse) on the anxiety response.

Summary

The EPM test has been a principal component of the anxiolytic drug screening framework for more than a decade. It has become so widely employed that not only are drugs routinely tested with it, but it has been one of the gold-standard tests to which comparison responses are often made. The data presented indicate that hormetic dose responses for anxiolytic effects have been routinely and replicatively reported in the EPM using a variety of animal models and by agents acting through a highly diverse range of receptor pathways.

LIGHT–DARK TEST

Historical Foundations

In 1980 Crawley and Goodwin proposed a new test to evaluate anxiolytic effects called the light–dark (L-D) test. It was designed, as the authors noted, to be simple, accurate, quick, with no need for prior animal training, as well as lacking assumptions relating to the model's pain threshold or appetite. The L-D test is founded on observations that even though mice and rats display strong tendencies to explore a novel environment, lighted open areas have aversive properties that inhibit this exploratory behavior. When given a choice between exploring a lighted open area or a dark enclosed one, the rodent will strongly favor the dark area. Anxiolytic agents would be expected to reverse this tendency.

With this as their theoretical framework, Crawley and Goodwin (1980) designed a test chamber with light and dark sections. The dark section was painted black and covered. The light section was painted white and made open to a light source. The dark section had an area 50% the size of the light area (i.e., 66.6% to 33.3% light–dark area ratio).

In their initial evaluation of the light–dark test system, Crawley and Goodwin (1980) employed male NIH albino mice in 10-min sessions/animal, following an ip administration of a

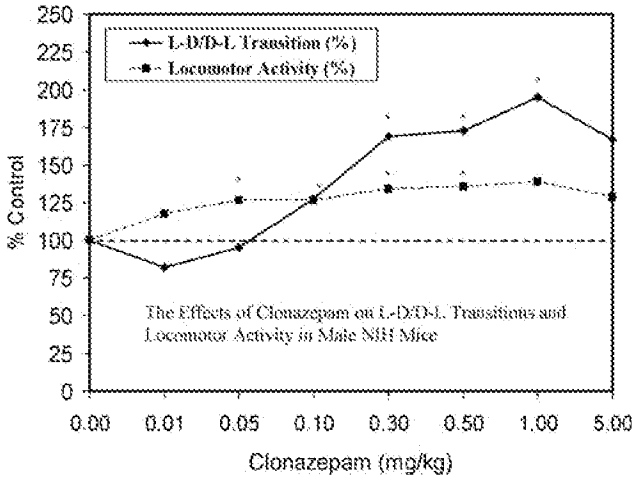

FIG. 4. The effects of clonazepam (benzodiazepine) on light–dark (L-D)/dark–light (D-L) transitions and locomotor activity in male NIH mice. *Significantly different from controls at $p < .05$. (Source: Crawley and Goodwin, 1980, Figures 2 and 3, p. 169).

test drug (i.e., two benzodiazepine drugs: clonazepam or chlordiazepoxide, tested separately) some 30 min prior to evaluation. The endpoints measured were the total number of transitions across the partition between light and dark chamber sections and total locomotor activity counts in both areas. A key provision in this test was to ensure that any positive decision of enhanced exploratory behavior (i.e., lowered anxiety) was not simply due to a general increase in locomotor activity.

In the testing of light–dark/dark–light transitions and locomotor activity, Crawley and Goodwin (1980) employed seven

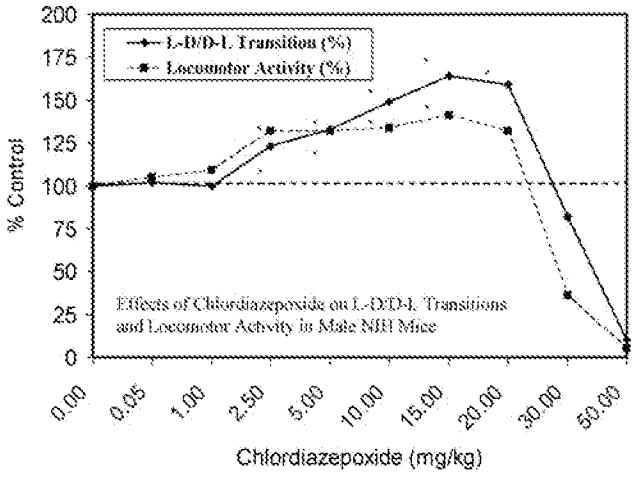

FIG. 5. Effects of chlordiazepoxide (benzodiazepine) on light–dark/dark–light transitions and locomotor activity in male NIH mice. *Significantly different from controls at $p < .05$. (Source: Crawley and Goodwin, 1980, Figures 2 and 3, p. 169).

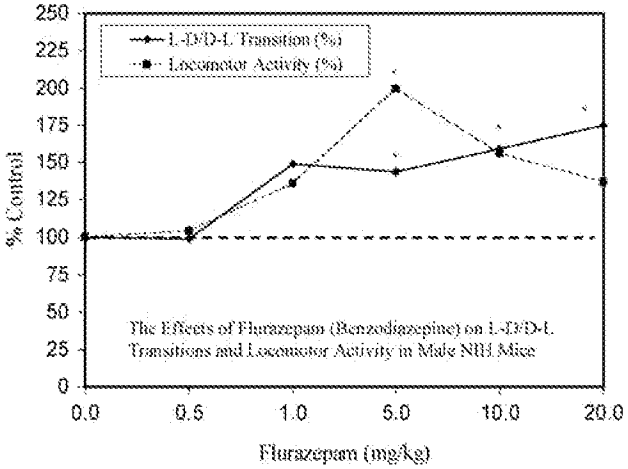

FIG. 6. Effects of flurazepam (benzodiazepine) on light–dark/dark–light transitions and locomotor activity in male NIH mice. *Significantly different from controls at $p < .05$. (Source: Crawley, 1981, Table 1, p. 696).

(500-fold dose range) and nine (1000-fold dose range) doses for clonazepam (Figure 4) and chlordiazepoxide (Figure 5), respectively. There was a dose-dependent increase in total locomotor activity and light–dark/dark–light transitions for both agents. Even though these drugs were effective in increasing locomotor activity in the light–dark test, they did not "appear to increase locomotor activity in a bare and undifferentiatied cage which was either uniformly illuminated or uniformly darkened," suggesting that these two drugs may be affecting exploratory behavior rather than having a generalized effect on motor activity. This conclusion was reemphasized in subsequent papers with several benzodiazepines (Figures 6–9) (Crawley, 1981, 1985; Crawley

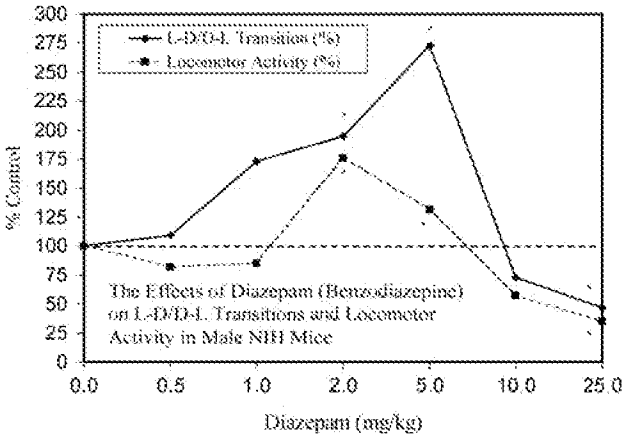

FIG. 7. Effects of diazepam (benzodiazepine) on light–dark/dark–light transitions and locomotor activity in male NIH mice. Note dose of 1.0 mg/kg replaced 0.5 mg/kg which was believed to have been made in error by authors. *Significantly different from controls at $p < .05$. (Source: Crawley, 1981, Table 1, p. 696).

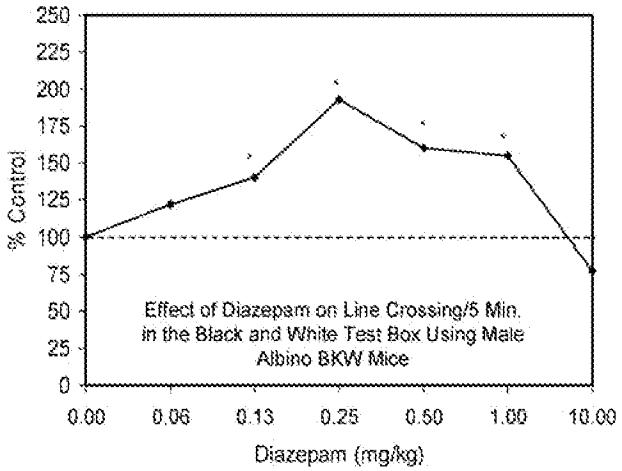

FIG. 8. The effect of diazepam on line crossing/5 mi. in the black and white test box using male albino BKW mice. *Significantly different from controls at $p < .05$. (Source: Costall et al., 1989, Figure 5, p. 782).

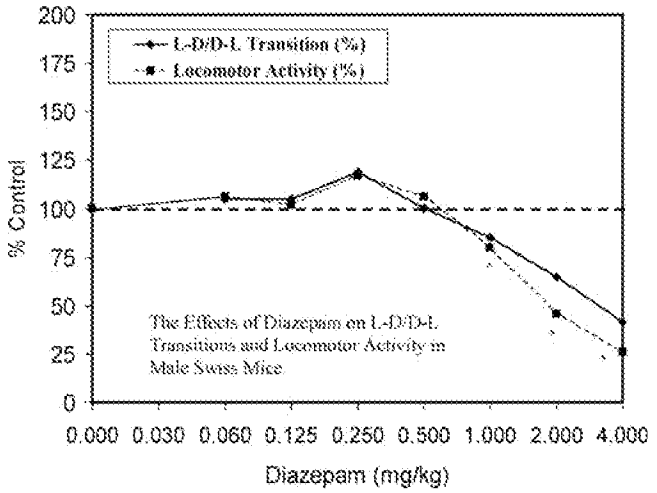

FIG. 10. Light–dark transitions and spontaneous locomotor activity of male Swiss mice after diazepam injections. *Significantly different from controls at $p < .05$. (Source: Hascoet and Bourin, 1998, Table 1, p. 647).

and Davis, 1982; Crawley and Goodwin, 1980; Crawley et al., 1984a,b; Belzung et al., 1987; Young and Johnson, 1991; Costall et al., 1989; Griebel et al., 2002a,b). Even though differentiating exploratory from locomotor activity was a critical methodological feature in the evaluation of potential anxiolytic agents, the testing within the basic, undifferentiated cage was quite limited, having been restricted to a single dose in the original Crawley and Goodwin (1980) paper. In the lights-off section there was a nonsignificant 12% increase in locomotor activity with chlordiazepoxide. A more rigorous testing of the dose-response relationship has been needed to evaluate a possible general locomotor response. This concern was emphasized with stronger documentation by Hascoet and Bourin (1998), who were able to associate light–dark/dark–light transitions with locomotor activity in a bare and undifferentiated cage in the testing of diazepam and alprozolam (Figures 10 and 11). Testing over the same dose range for both endpoints (i.e., transitions and locomotor activity) is critical to ruling in or out a locomotor or exploratory interpretation.

Another contentious conclusion of the original Crawley and Goodwin (1980) paper related to the time spent in the darkened one-third of the chamber. The only significant increase was seen at the 50-mg/kg dose with chlordiazepoxide. However, this was a dose that was associated with sedation (i.e., highest dose tested in Figure 5). The authors concluded that even

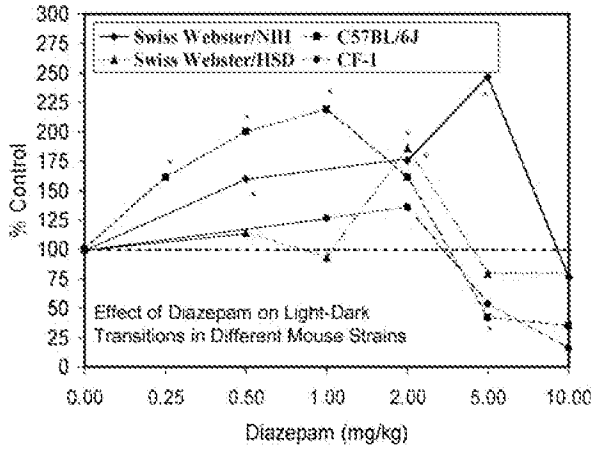

FIG. 9. Effect of diazepam on light–dark transitions in different mouse strains. *Significantly different from controls at $p < .01$. (Source: Crawley and Davis, 1982, Figure 1, p. 610).

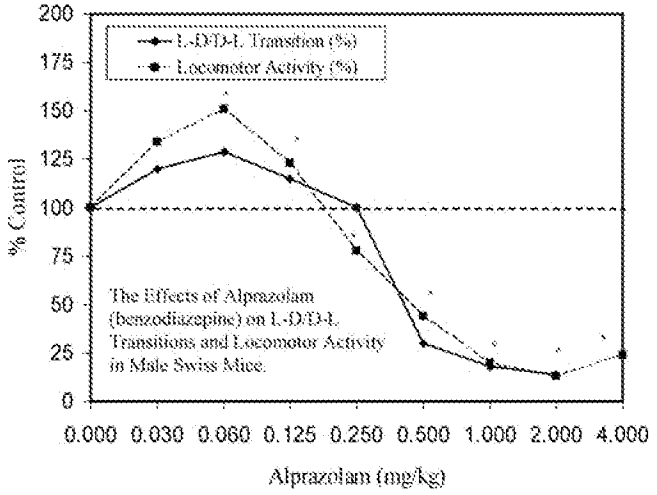

FIG. 11. Light–dark transitions and spontaneous locomotor activity of male Swiss mice after alprazolam (benzodiazepine) injections. *Significantly different from controls at $p < .05$. (Source: Hascoet and Bourin, 1998, Table 1, p. 647).

though these two drugs (i.e., clonazepam and chlordiazepoxide) did not seem to affect the dark preference, they increased locomotor-linked exploratory behavior in response to some property of the two chamber testing framework. Thus, the key practical finding was that a single endpoint (i.e., transitions across the barrier between the light and dark sections) could be a simple, accurate, and efficient index to predict anxiolytic effects.

While this methodologic framework was a potentially valuable development for anxiolytic compound screening, several important dose-response features should be highlighted. Both light–dark/dark–light transitions and locomotor activity followed an hormetic-like biphasic dose response relationship (Figures 4 and 5). Even though the light–dark transition and locomotor activities in the original Crawley and Goodwin (1980) paper were highly correlated they have some quantitative differences, which may have biological/interpretative significance. For example, relative to a value of 100% the transition endpoint response was consistently greater than the locomotor responses for both compounds (Figures 4 and 5), an observation that was further strengthened and extended in Crawley (1981). This indicates that there is more "activity" at the light–dark/dark–light interface than over other areas, suggesting that the locomotor activity is more "concentrated" at the interface of the light–dark transition zone. This further implies that a substantial proportion of the increased locomotor activity is directly accounted for by light–dark/dark–light transitions, which supports an exploratory interpretation (i.e., anxiolytic response).

In the case of clonazepam, the increase in locomotor activity occurred at a 10-fold lower concentration than did the increase in transitions (Figure 4). Even though these endpoints are highly correlated they can be at least partially differentiated by the magnitude of response and dose response range over which they act, findings not addressed by Crawley and Goodwin (1980) or in later papers by Crawley. Such observations were experimentally confirmed (but not discussed) by Crawley (1981) as she extended the initial findings to a broader range of benzodiazepine drugs. The case for exploratory behavior was partially enhanced by data showing a positive (0.72) correlation between transitions and frequency of rearing position associated with the exploratory sniffing of the cage (Crawley, 1981).

The experimental findings in the light–dark test within the Crawley research papers is highly reproducible, based on direct quantitative comparisons of the clonazepam and chlordiazepoxide. This was the case for the transition and locomotor activity endpoints. Thus, the hormetic dose-response features seem to be a highly credible finding in this system. Similar replications have been reported with diazepam as well (Figures 7–9) (Crawley and Goodwin, 1980; Crawley et al., 1981). Likewise, the capacity for diazepam to produce an hormetic-like biphasic dose response with generally similar quantitative features as seen by the magnitude of stimulatory response was reported by

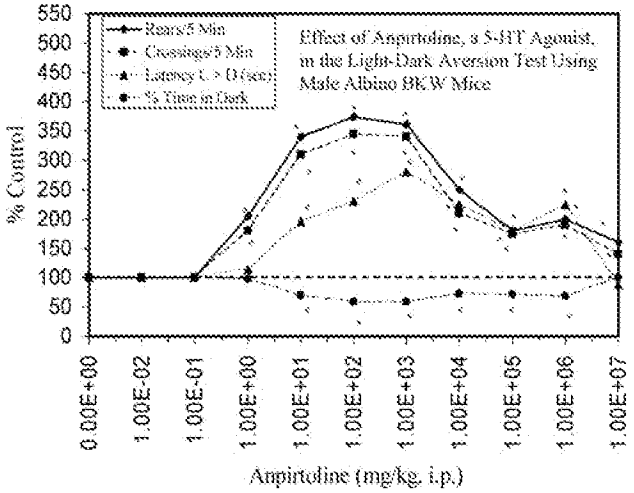

FIG. 12. Behavioral activity of anpirtonline, a 5-HT agonist, assessed in the light–dark mouse aversion test using male albino BKW mice. *Significantly different from controls at $p < .05$. (Source: Metzenauer et al., 1992, Figure 1, p. 529).

Crawley and Davis (1982) with four mouse strains (i.e., Swiss-Webster/NIH, C57Bl/6J, Swiss Webster/HSD1, and CF-1) (Figure 9).

5-HT Pathway for Anxiolytic Responses

Of interest was the report of Metzenauer et al. (1992) that anpirtoline increased the initial latency of male albino BKW mice to move from the light to dark compartment and decreased the proportion of time spent and the number of rearings and line crossings in the dark compartment of the test system. The anxiolytic-like effect in the light–dark aversion test occurred over a 10^5- to 10^6-fold dose range (Figure 12). This is an extraordinary dose range of activity compared to other anxiolytic agents in this test system. Since this agent is believed to act via the 5-HT receptor, this may be an interesting model system to better understand factors affecting the quantitative features of the dose response. Hormetic dose responses were also reported with the 5-HT_{1A} agonist ipsapirone and the 5-HT_3 receptor antagonist ondansetron in female ICR-DUB mice (Young and Johnson, 1991), observations that were consistent with those reported by Rex et al. (1998) with male Wistar rats.

Dopamine Pathway for Anxiolytic Responses

Hormetic biphasic dose responses have also been reported with male CD Sprague-Dawley rats in the light–dark test (Pich and Samanin, 1986) with buspirone, haloperidol, and sulpiride (Figures 13 and 14), using light–dark transitions as the endpoint. Since prior research had revealed that low doses of sulpiride and buspirone act as dopamine antagonists at DA autoreceptors (McMillen et al., 1983; Tissari et al., 1979) the decreased anxiety was hypothesized as being mediated via the DA receptor. This

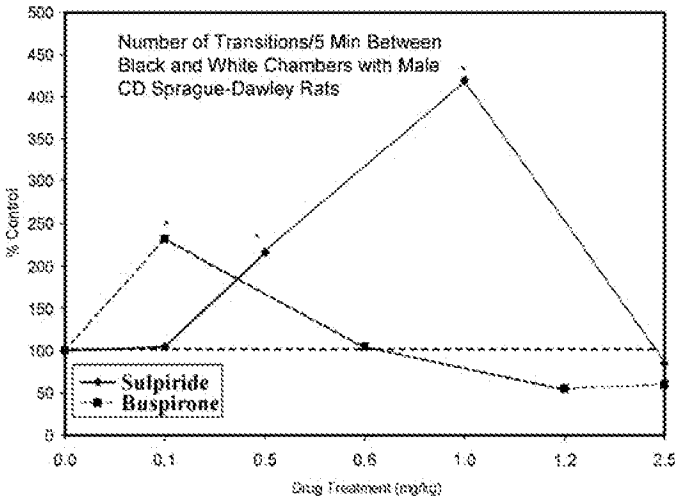

FIG. 13. Number of transitions/5 min between black and white chambers (BWT) in the two-compartment exploratory test after injection of buspirone, a non-benzodiazepine compound, and dopamine receptor agonist sulpiride with male CD Sprague-Dawley rats. *Significantly different from controls at $p < .05$. (Source: Pich and Samanin, 1986, Figure 2, p. 128).

was confirmed in subsequent experiments with the DA agonist apomorphine, which eliminated the anxiolytic effects of buspirone and sulpiride, suggesting that selected antagonists at DA autoreceptor may possess disinhibitory properties. Likewise, a similar hormetic-like biphasic dose response (Figure 15) was reported with the centrally acting antitussive fominoben (Crawley et al., 1984a).

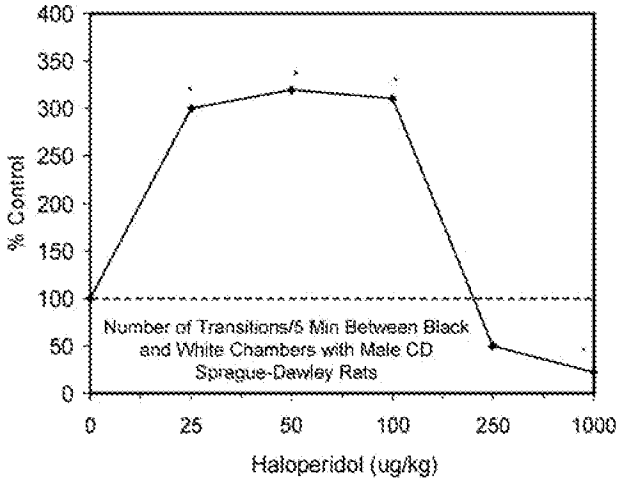

FIG. 14. Number of transitions/5 min between black and white chambers (BWT) in the two-compartment exploratory test after injection of the neuroleptic agent, haloperidol, with male CD Sprague-Dawley rats. *Significantly different from controls at $p < .05$. (Source: Pich and Samanin, 1986, Figure 2, p. 128).

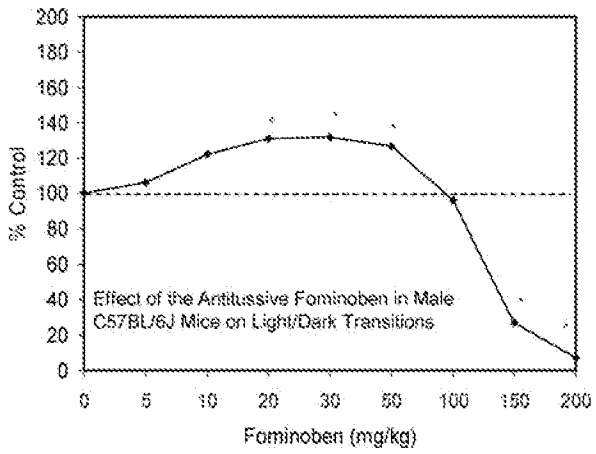

FIG. 15. Anxiolytic actions of the antitussive fominoben in male C57BL/6J mice on light–dark transitions. *Significantly different from controls at $p < .05$. (Source: Crawley et al., 1984a, Figure 2, p. 279).

Anxiogenic Versus Anxiolytic Responses

Anxiogenic compounds can also be evaluated in the light–dark test. In such testing, hormetic-like dose responses were also reported with similar quantitative features of the dose-response relationship (Kilfoil et al., 1989; Griebel et al., 2002a, 2002b). For example, the anxiogenic agent FG-7142 biphasically increased time spent in the dark area (Figure 16), a sign of increased anxiety (Kilfoil et al., 1989). Similarly, capacity to enhance anxiogenic behavior at low doses was reported by Sanchez and Meier (1997) with selective serotonin reuptake inhibitors (SSRI) (Figures 17 and 18) and Griebel et al. (2002a) with corticotrophin-releasing factor (CRF_1) receptor antagonists

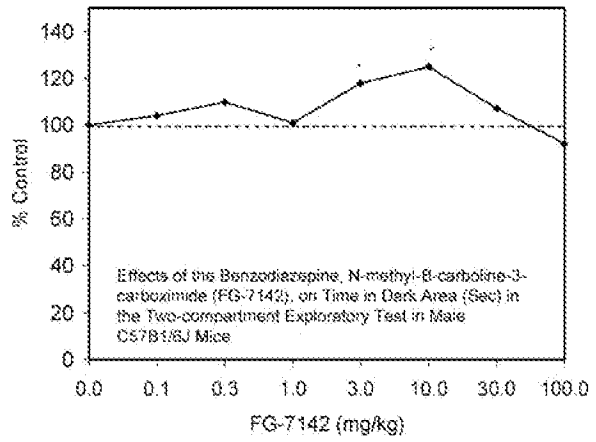

FIG. 16. Effects of *N*-methyl-B-carboline-3-carboximide (FG-7142), a benzodiazepine, on time in dark area (seconds) in the two-compartment exploratory test in male C57B1/6J mice. *Significantly different from controls at $p < .05$. (Source: Kilfoil et al., 1989, Table 1, p. 902). Note that this behavior represents an anxiogenic response.

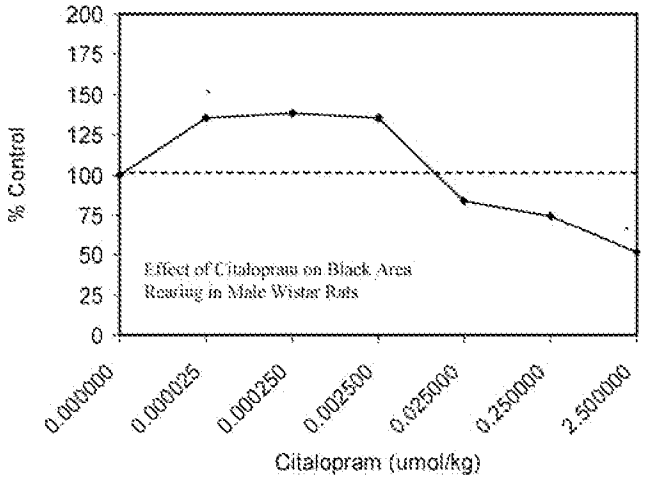

FIG. 17. Effect of citalopram, a selective serotonin reuptake inhibitor (SSRI), on exploratory behavior of male Wistar rats in a two compartment black and white test box. *Significantly different from controls at $p < .05$. (Source: Sanchez and Meier, 1997, Table 4, p. 201). Note that this behavior in the low dose zone represents an anxiogenic response.

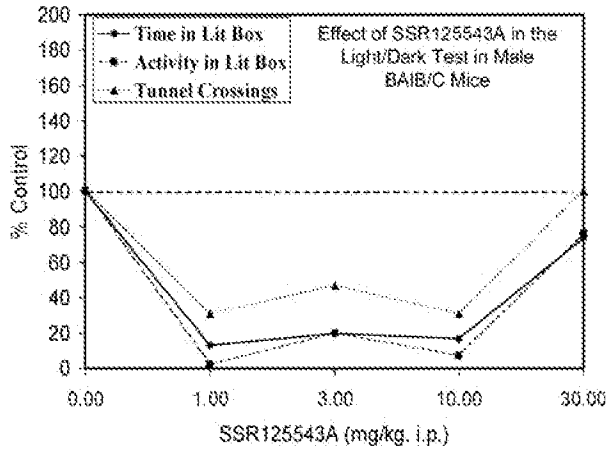

FIG. 19. Effects of SSR125543A (2-aminothiazole derivative), a novel corticotrophin-releasing factor (CRF_1) receptor antagonist, in the light/dark test in male BAlB/C mice. (Source: Griebel et al., 2002a, Table 3, p. 338). In the low dose zone the behavior represents an anxiogenic response.

(Figures 19 and 20). These findings confirm the generalized nature of hormetic dose-response relationships accounting for both anxiolytic and anxiogenic effects in the light–dark test.

Summary

This assessment of the light–dark test for anxiolytic behavior indicates that hormetic-like biphasic dose responses were commonly seen across a broad range of agents, including representative benzodiazepine drugs, 5-HT agonists, dopaminergic agents, CRF_1 antagonists, and the neuroleptic haloperidol. Despite the widely differing receptor-mediated mechanisms leading to the anxiolytic (and anxiogenic) behavior, quantitative features of these respective dose responses were similar, with maximum stimulatory response typically less than twice the control value while the stimulatory responses spanned a concentration range generally encompassing 10- to 50-fold immediately beneath the threshold. However, as noted earlier, the unusually broad stimulatory response range of the 5-HT agonist anpirtoline was extraordinary and worthy of further mechanistic clarification.

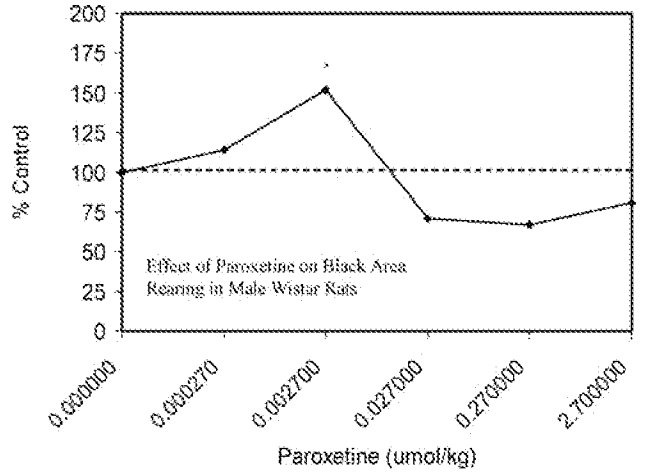

FIG. 18. Effect of paroxetine, a selective serotonin reuptake inhibitor (SSRI), on exploratory behavior of male Wistar rats in a two compartment black and white test box. *Significantly different from controls at $p < .05$. (Source: Sanchez and Meier, 1997, Table 4, p. 201). Note that this behavior in the low dose zone represents an anxiogenic response.

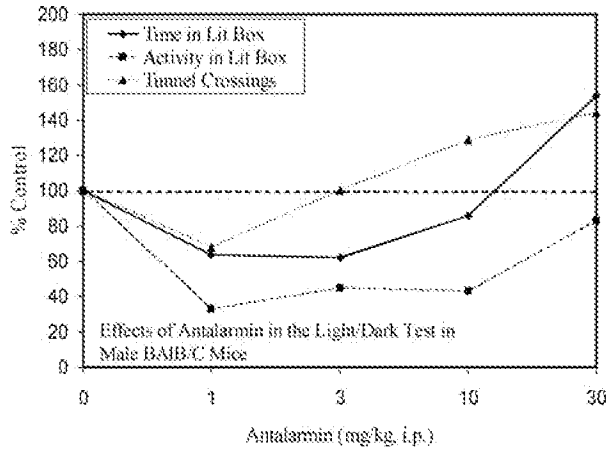

FIG. 20. Effects of antalarmin, a CRF_1 receptor antagonist, in the light/dark test in male BAlB/Cmice. (Source: Griebel et al., 2002a, Table 3, p. 338). In the low dose zone the behavior represents an anxiogenic response.

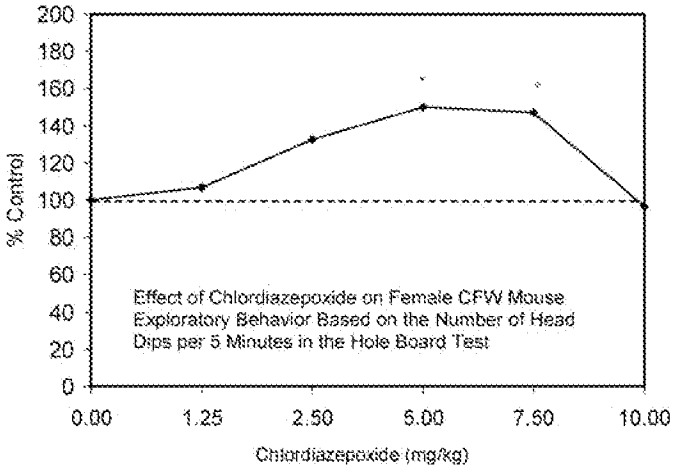

FIG. 21a. The effect of chlordiazepoxide on female CFW mouse exploratory behavior in the hole board test. Number of head dips/5 min. *Significantly different from controls at $p <$ .05. (Source: Nolan and Parkes, 1973, Table 1, p. 283).

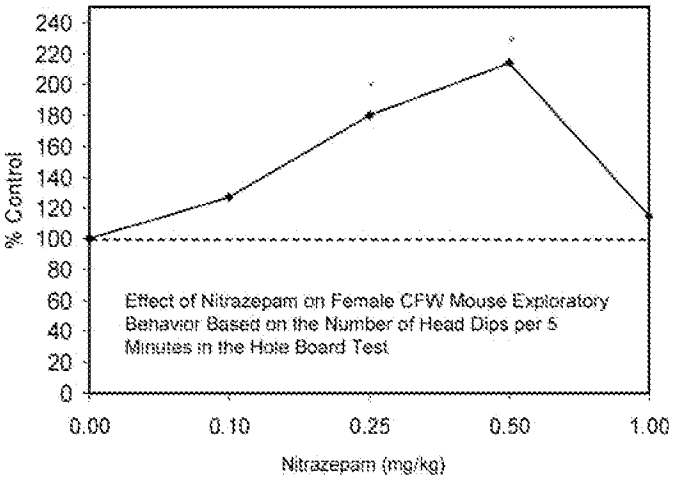

FIG. 21c. The effect of nitrazepam on female CFW mouse exploratory behavior in the hole board test. Number of head dips/5 min. *Significantly different from controls at $p < .05$. (Source: Nolan and Parkes, 1973, Table 1, p. 283).

HOLE-BOARD TEST

The hole-board test, which was developed by Boissier and Simon (1962, 1964) nearly a half century ago, provides a simple means to assess the response of an animal (i.e., mouse) to an unfamiliar setting. A typical hole-board uses the 40-cm-square and 1.8-cm-thick board with 16 equally spaced holes 3 cm in diameter. It is mounted on four 2.5-cm legs and placed on a gray floor in a cubicle with three gray painted walls with the observer seated near the floor at the window side. Each mouse is assessed by placing it singly in the center of the board facing away from the observer. A mouse is determined to have made a head dip if both ears go below the top of the hole. Each hole is numbered so that the number of times a mouse explores a specific hole may be counted.

In 1973 Nolan and Parkes assessed the effects of several benzodiazepines on exploratory activity in female mice of the CFW strain (Figure 21, a–e) within a four- or five-dose treatment protocol. Drugs were administered ip 30 min prior to exposure to the board, which was for 5 min duration. Five of the six drugs tested displayed a low-dose stimulatory response and a return to control or below control values at higher doses. The only drug failing to show a significant increase at any dose (0.1–12.5

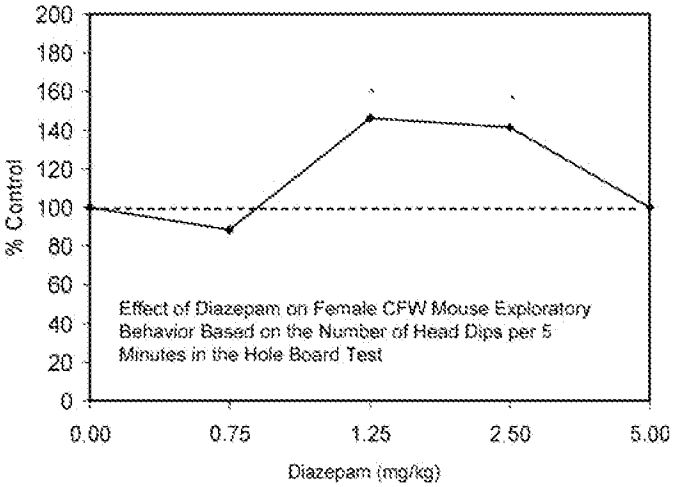

FIG. 21b. The effect of diazepam on female CFW mouse exploratory behavior in the hole board test. Number of head dips/5 min. *Significantly different from controls at $p < .05$. (Source: Nolan and Parkes, 1973, Table 1, p. 283).

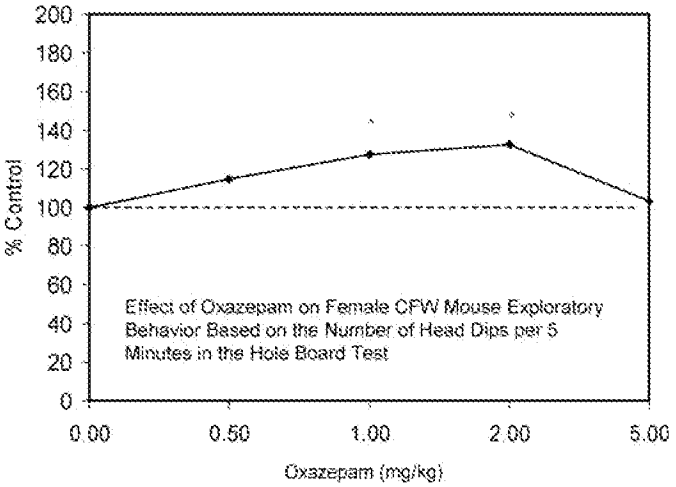

FIG. 21d. The effects of oxazepam on female CFW mouse exploratory behavior in the hole board test. Number of head dips/5 min. *Significantly different from controls at $p < .05$. (Source: Nolan and Parkes, 1973, Table 1, p. 283).

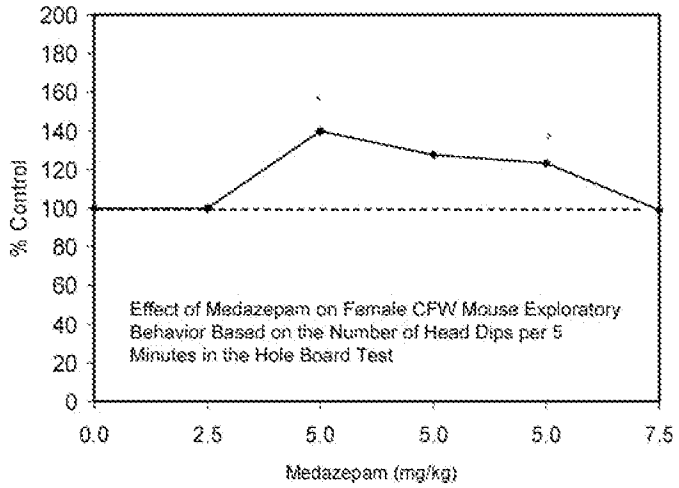

FIG. 21e. The effect of medazepam on female CFW mouse exploratory behavior in the hole board test. Number of head dips/5 min. Note that three replications at 5.0 mg/kg were reported. *Significantly different from controls at $p < .05$. (Source: Nolan and Parkes, 1973, Table 1, p. 283).

mg/kg) was flurazepam, for which the data were not shown. The dose-response relationships were similar, with maximum stimulation of 213% for nitrazepam, with the remaining four drugs being between 130 and 160%. The stimulatory ranges, though difficult to precisely determine, were modest, being a dose range of about 4-fold for chlordiazepoxide and diazepam and a factor of about 2 for medazepam, while nitrazepam was greater than a 10-fold range and oxazepam had a stimulatory range between 5- and 10-fold. The research of Nolan and Parkes (1973) has been

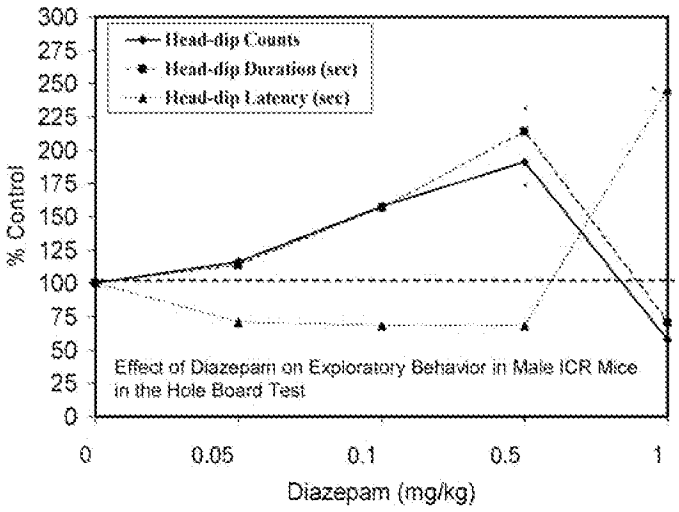

FIG. 22. Effect of diazepam on exploratory behavior in male ICR mice tested in the hole-board test. *Significantly different from controls at $p < .05$. (Source: Takeda et al., 1998, Figure 2, p. 23).

replicated many times and extended to modified test methods and other animal models, as seen with the research of Takeda et al. (1998) using the male ICR mice (Figure 22), as well as in models with medical conditions such as diabetes (Kamei et al., 2001).

It is interesting to note that when chlordiazepoxide was administered to mice prior to a second exposure to the hole-board, no increase of exploratory activity was found for doses that were effective during a first exposure test. However, activity was reduced by high sedative doses of the chlordiazepoxide as occurred after the first dose. These findings were hypothesized as showing that benzodiazepines may act "only to remove a factor that limits exploratory activity of mice in a novel situation." In effect, these drugs appear to enhance exploratory activity in the novel environment by a release of novelty suppression behavior (Soubrie et al., 1977; Gardner and Piper, 1982).

A range of benzodiazepine drugs, including clobazam, diazepam, nitrazepam, and flunitrazepam, enhanced exploration (head dipping) with mice (naive male CD-1) in the hole-board test at nonsedative doses (Gardner and Piper, 1982). The dose-response patterns followed the hormetic biphasic pattern reported by Nolan and Parkes (1973) with respect to the magnitude of stimulation and the dose stimulation range. Locomotion patterns also followed a similar dose-response scheme as the exploratory behavior. However, using a chronic dosing strategy, Gardner and Piper (1982) demonstrated that the increase in exploratory behavior remained, yet the locomotor effects declined, thereby showing a tolerance for the locomotor response. Based on follow-up experiments that partially differentiated locomotor activity from exploratory behavior [e.g., urethane, muscimol (stimulation of GABA receptors), and amino-oxyacetic acid (AOAA)], and with an inhibitor of GABA transaminase, sodium valproate and γ-acetylenic GABA (GAG) (inhibitors of GABA catabolism), these findings suggest that anxiolytic effects have a certain type of specificity resulting from selective enhancement of brain GABA transmission.

More recent studies by Leung et al. (2003) have more tightly linked drug-enhanced anxiolytic events to the $GABA_A$-receptor benzodiazepine site (BDS) using both the hole-board (Figure 23) and the EPM tests (Figure 24). In this study *d*, *l*-tetrahydrapalmatine (*dl*-THP) (Figure 25), a naturally occurring alkaloid, induced anxiolytic effects that closely resembled hormetic dose responses. In follow-up experimentation, specific antagonists for the BDZ effect completely prevented the *dl*-THP-induced anxiolytic effect using the EPM test. These findings clearly established that the *dl*-THP induced anxiolytic effect is mediated via the benzodiazepine receptor.

While these experiments have attempted to better define the role of GABA-mediated neurotransmission on the drug exploratory behavior of selected anxiolytic benzodiazepine drugs, they commonly display the hormetic-like biphasic dose response for most of the drugs tested and for both exploratory

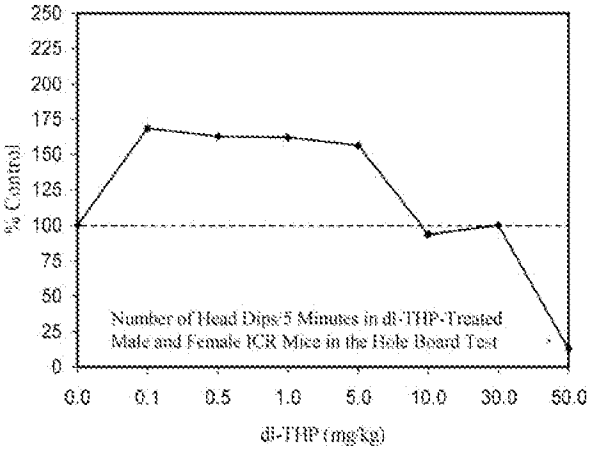

FIG. 23. Number of head dips/5 min in dl-THP-treated male and female ICR mice in the hole board test. *Significantly different from controls at $p < .05$. (Source: Leung et al., 2003, Figure 3, p. 777).

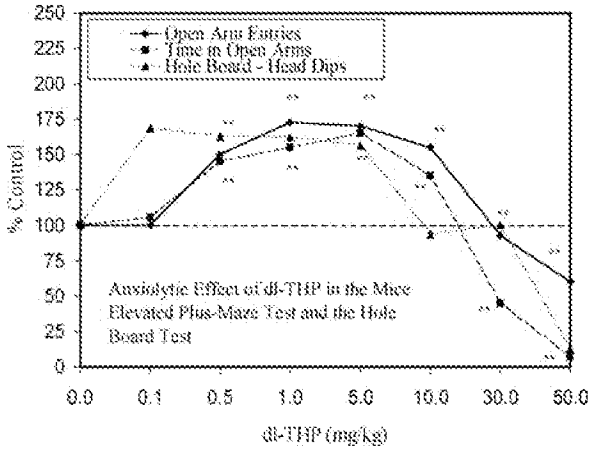

FIG. 25. Comparison of dl-THP on mice in the elevated plus-maze test and in the hole board test (combined Figures 23 and 24). *Significantly different from controls at $p < .05$. (Source: Leung et al., 2003, Figures 2 and 3, p. 777).

and locomotor endpoints, whether they could be partially mechanistically differentiated or not.

OPEN FIELD STUDY

The open field test was developed by Hall (1934) to assess the emotionality of rats. The test involves subjecting the animal to an unknown new environment from which it is not allowed to escape due to the presence of surrounding walls. The experimental system is comprised of a brightly illuminated circular arena (1.2 m diameter) that is enclosed by a wall 0.45 m high. In the test an individual rat is placed in the outer ring of the open field and observed for usually 2 min, a process that is repeated in daily trials. Rats have been commonly food deprived for 24 to 48 h prior to testing. If the animals did not eat they were viewed as "emotional." In general, "emotional" rats were found

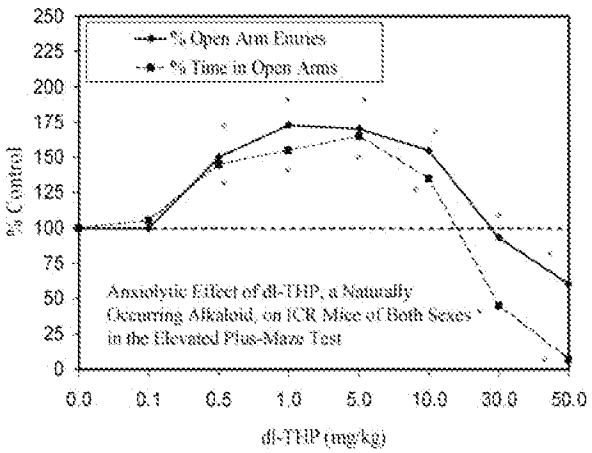

FIG. 24. Anxiolytic effect of dl-THP on male and female ICR mice in the elevated plus-maze test. *Significantly different from controls at $p < .05$. (Source: Leung et al., 2003, Figure 2, p. 777).

to have fewer entries into the central part of the arena and had higher levels of defecation (Prut and Belzung, 2003). Over the intervening years the open field test became extremely popular in animal psychology, with numerous modifications (different shape of environment, lighting intensity/color, other objects in arena, etc.) made to the original design, depending on investigator interests.

In most recent studies, the data typically collected include information on horizontal locomotion, frequency of rearing, grooming, and location of activity (central vs. periphery). Of relevance to the present analysis is that increases of time spent in the central part of the arena, and in the central part/total arena ratio, and a decrease of the latency for entering the central part are traditionally interpreted as anxiolytic behaviors.

The open field test has been used to assess dose-response relationships of anxiolytic drugs using male rats (Christmas and Maxwell, 1970; Britton and Britton, 1981; Stefanski et al., 1993; Rex et al., 1998; Horvath et al., 1992). Rex et al. (1998) assessed the effects of 10 known anxiolytic drugs for their capacity to affect early activity in the modified open field test within a dose-response context (Figure 26, a–j). In this study the male Wistar rats were food deprived 20 h before testing. Food was placed in the center of the open field as pellets in a petri dish. Each rat was initially placed in one area of the unfamiliar open field, facing the center. Each rat was observed for 5 min and the time to the initial feeding was recorded. All drugs displayed a hormetic-like U-shaped dose response, confirming the earlier recognition of their anxiolytic properties. When deprived rats were given access to food in familiar cages the drugs had no effect on food consumption, further supporting the anxiolytic interpretation. Since the study design included observing the rats for a duration of latency that was capped at 300 s, this prevented a determination of the actual latency, which may have exceeded the 300-s time cap at the highest dose for most, if not all, drugs tested

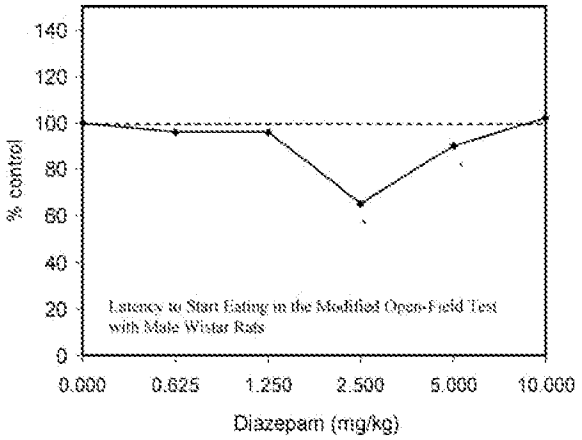

FIG. 26a. Assessment of multiple drugs in the modified open field test using the male Wistar rat. *Significantly different from controls at $p < .05$. (Source: Rex et al., 1998). Diazepam (Source: Rex et al., 1998, Table 1, p. 679).

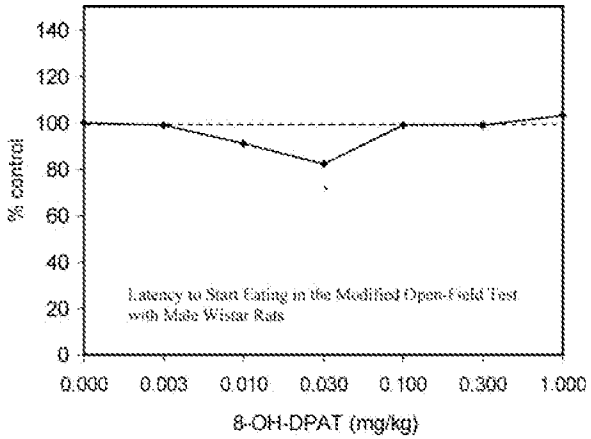

FIG. 26b. 8-OH-DPAT. (Source: Rex et al., 1998, Table 1, p. 679).

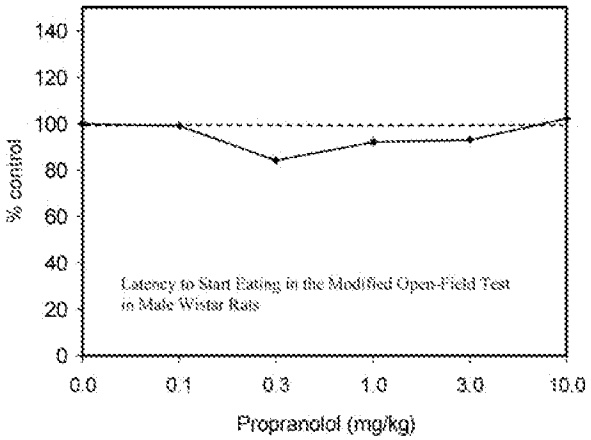

FIG. 26c. Propranolol. (Source: Rex et al., 1998, Table 1, p. 679).

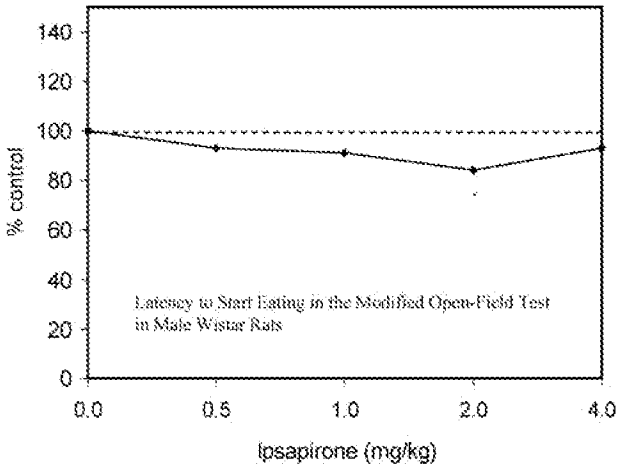

FIG. 26d. Ipsapirone. (Source: Rex et al., 1998, Table 1, p. 679).

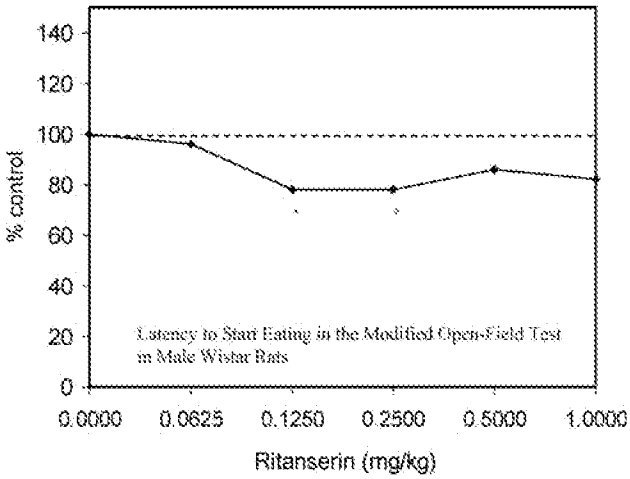

FIG. 26e. Ritanserin. (Source: Rex et al., 1998, Table 1, p. 679).

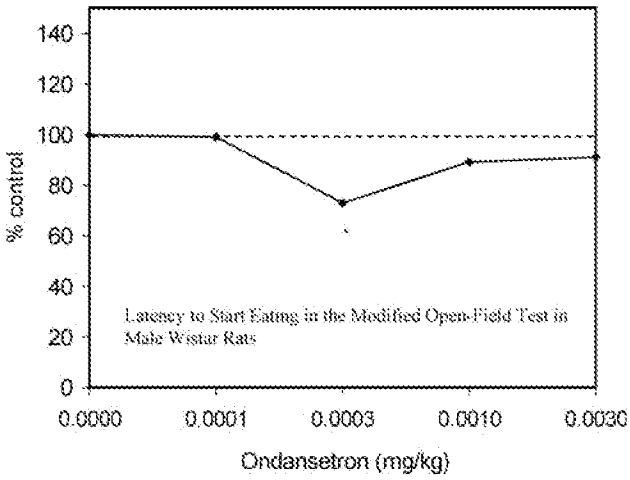

FIG. 26f. Ondansetron. (Source: Rex et al., 1998, Table 1, p. 679).

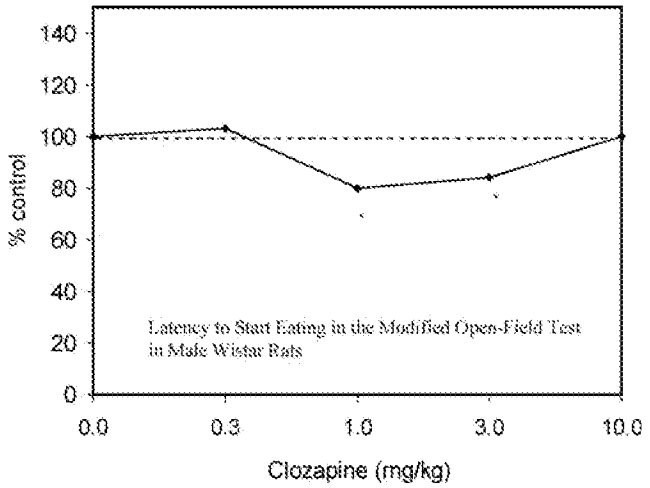

FIG. 26g. Clozapine. (Source: Rex et al., 1998, Table 1, p. 679).

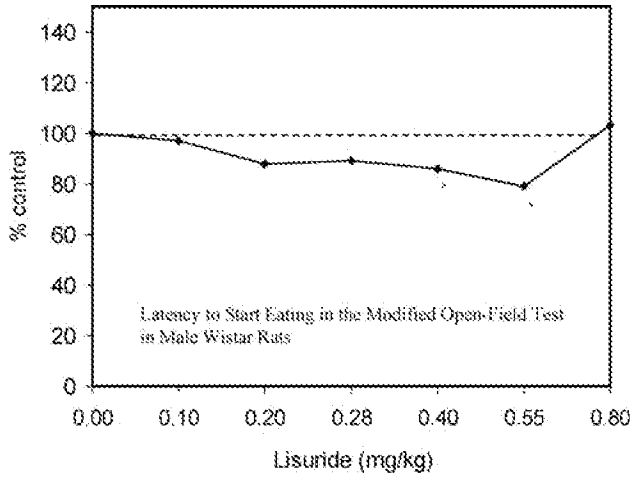

FIG. 26h. Lisuride. (Source: Rex et al., 1998, Table 1, p. 679).

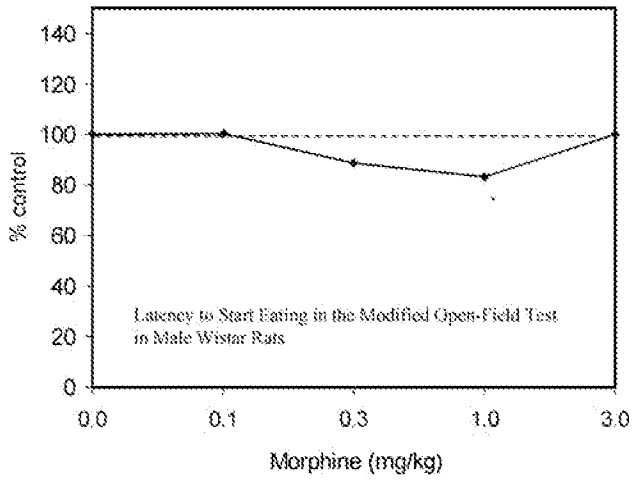

FIG. 26i. Morphine. (Source: Rex et al., 1998, Table 1, p. 679).

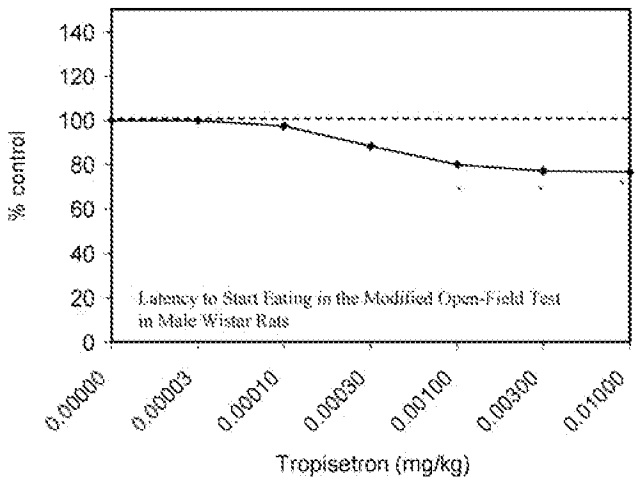

FIG. 26j. Tropisetron. (Source: Rex et al., 1998, Table 1, p. 679).

based on existing dose-response trends. Thus, this feature in the study design probably precluded showing genuine J-shaped dose responses.

In similar studies, anxiolytic-like effects of 5-HT_{1A} receptor agonists were shown to display hormetic biphasic dose responses using male Wistar rats. The effects, which appeared to be mediated in the hippocampus, were consistent across three endpoints (i.e., motor activity, central area entry, stay time in the central area) (Figure 27, a–d) (Stefanski et al., 1993).

Hormetic-like biphasic dose responses were also reported by Horvath et al. (1992, 2000) for two 2,3-benzodiazepines (GYKI

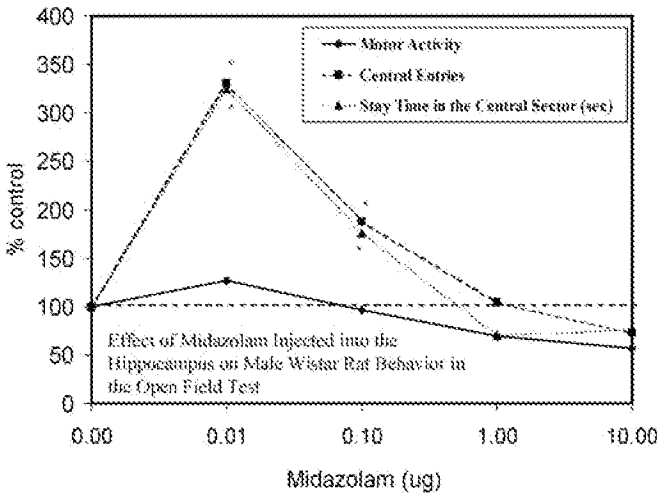

FIG. 27a. Effect of midazolam injected into the hippocampus on male Wistar rat behavior in the open field test. *Significantly different from controls at $p < .05$. (Source: Stefanski et al., 1993, Table 2, p. 981).

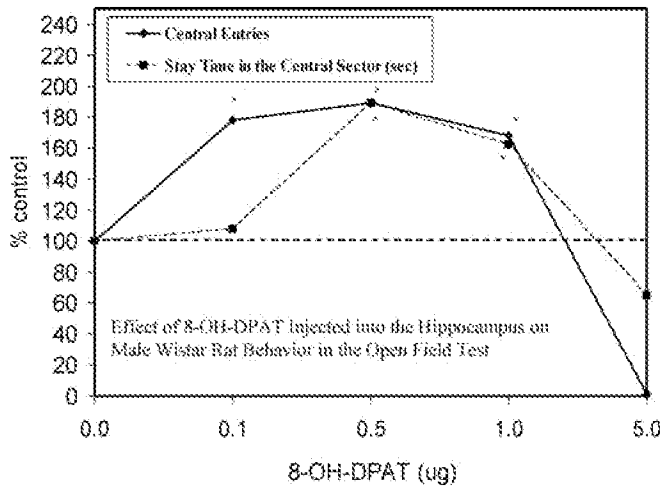

FIG. 27b. Effect of 8-OH-DAPT injected into the hippocampus on male Wistar rat behavior in the open field test. *Significantly different from controls at $p < .05$. (Source: Stefanski et al., 1993, Table 2, p. 981).

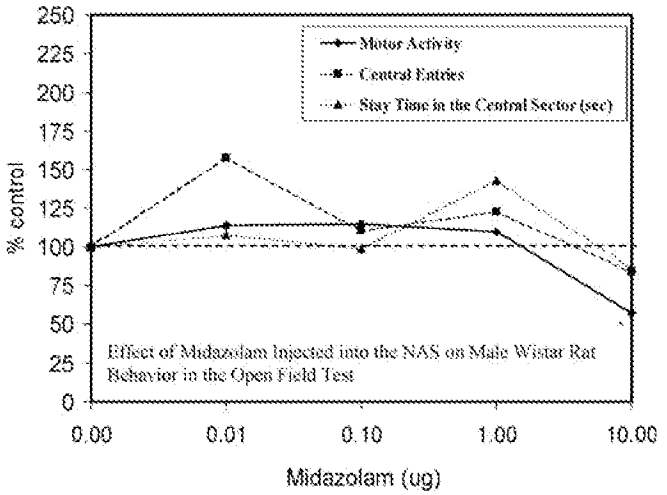

FIG. 27d. Effect of midazolam injected into the nucleus accumbens septi (NAS) on male Wistar rat behavior in the open field test. *Significantly different from controls at $p < .05$. (Source: Stefanski et al., 1993, Table 2, p. 981).

51 189 (1-(3-chlorophenyl)-4-methyl-7,8-dimethoxy-5H-2,3-benzodiazepine) and GYKI 52 322 (1-(4-aminophenyl)-4-methyl-7,8-dimethoxy-5H-2,3-benzodiazepine) as well as with chlordiazepoxide and buspirone. The quantitative features of the four drugs were consistent with the hormetic dose response (Figure 28, a–d).

The present analysis identified numerous agents evaluated in the open field test displaying hormetic-like biphasic dose-response relationships. Among the agents showing such biphasic dose responses were representatives of the benzodiazepines (e.g., 2,3-BZD and 1,4-BDZ), three different types of 5-HT ligands (i.e., 5-HT_{1A}, 5-HT_2 and 5-HT_3 antagonists), dopaminergic agents, and opiates. In general, the quantitative features of their dose-response relationships were similar, for example, with maximum response typically about 30–60% greater than the control with a concentration response range of about 5- to 20-fold immediately beneath the zero equivalent point (i.e., the traditional threshold). Consequently, even though a highly diverse set of drugs acting via different mechanisms induced the same type of anxiolytic behavior in the open field test, the quantitative

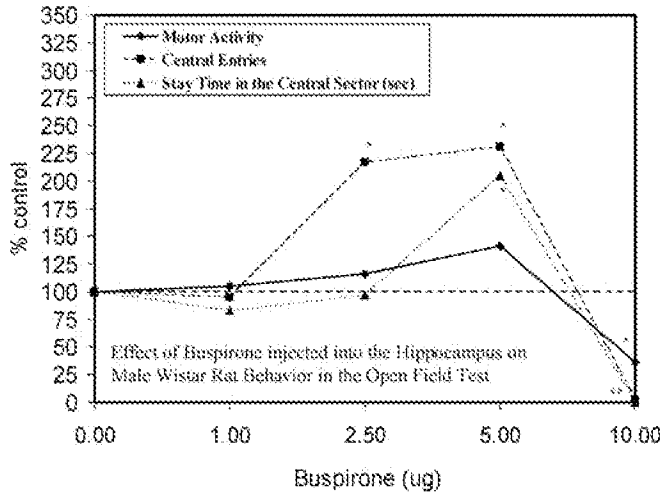

FIG. 27c. Effect of buspirone injected into the hippocampus on male Wistar rat behavior in the open field test. *Significantly different from controls at $p < .05$. (Source: Stefanski et al., 1993, Table 2, p. 981).

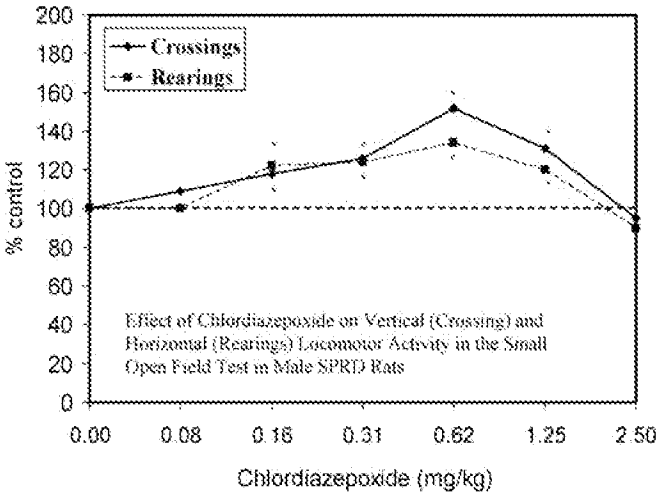

FIG. 28a. Effect of chlordiazepoxide on vertical (crossing) and horizontal (rearings) locomotor activity in the small open field test in male SPRD rats. * Significantly different from controls at $p < .05$. (Source: Horvath et al., 1992, Figure 3, p. 158).

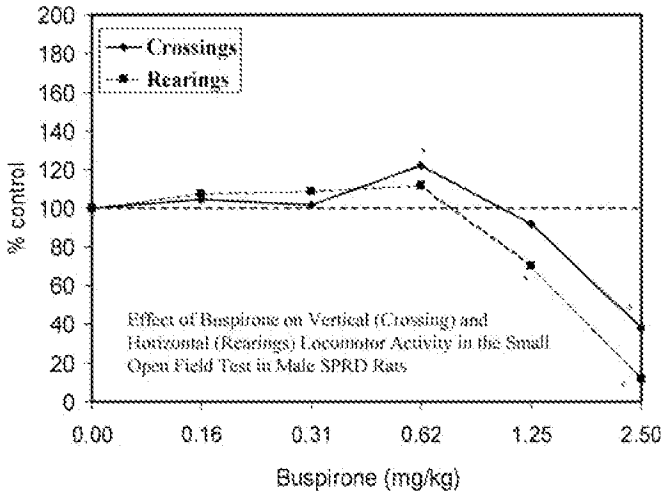

FIG. 28b. Effect of buspirone on vertical (crossing) and horizontal (rearings) locomotor activity in the small open field test in male SPRD rats. *Significantly different from controls at $p < .05$. (Source: Horvath et al., 1992, Figure 3, p. 158).

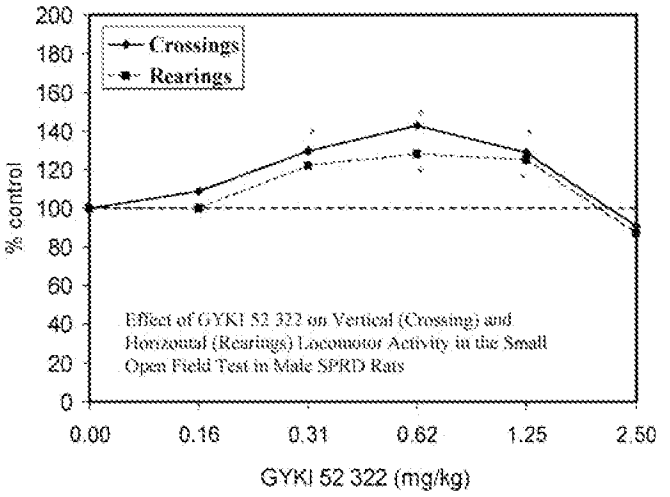

FIG. 28d. Effect of GYKI 52 322 (1-(4-aminophenyl)-4-methyl-7,8-dimethoxy-5*H*-2,3-benzodiazepine) on vertical (crossing) and horizontal (rearings) locomotor activity in the small open field test in male SPRD rats. *Significantly different from controls at $p < .05$. (Source: Horvath et al., 1992, Figure 3, p. 158).

features of the dose responses were similar, being consistent with that reported for numerous other hormetic dose responses that are independent of agent, biological model, and endpoint measured.

FOUR-PLATES TEST

In 1966 a metal plate-crossing test was introduced by Slotnick and Jarvik as a way to measure passive avoidance conditioning in mice. The equipment consisted of a box with a floor comprised of four metal plates. The testing procedure involved each mouse being placed onto one of the floor plates. The mouse was then allowed 15 s of exploration time. During the next 60 s the number of times the mouse crossed from one plate to another with all 4 feet was recorded. In the course of assessing the effects of drugs on passive avoidance via the four plate test (FPT), Boissier et al. (1968) noted that drugs used to reduce anxiety also reduced passive avoidance. These observations became relevant to the study of anxiety since an increase of the locomotor component of exploration could be interpreted as a response to novelty.

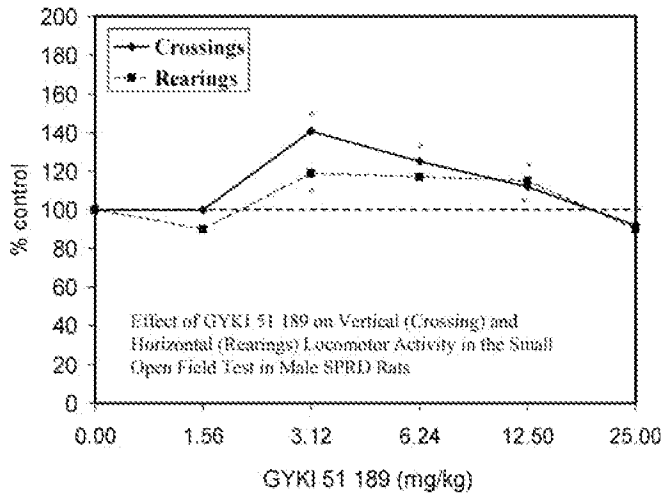

FIG. 28c. Effect of GYKI 51 189 (Girisopam) on vertical (crossing) and horizontal (rearings) locomotor activity in the small open field test in male SPRD rats. *Significantly different from controls at $p < .05$. (Source: Horvath et al., 1992, Figure 3, p. 158).

Since the original paper of Slotnick and Jarvik (1966), the FPT has become widely used for the screening of possible anxiolytic-acting drugs and testing a broad variety of investigator-generated hypotheses relating to research methods, study design, and mechanism assessment. Of particular importance to the present FPT evaluation is that on a number of occasions robust study designs with sufficient numbers of doses and a broad dose range have been employed that can be used to evaluate hormetic dose-response hypotheses.

The most extensive initial screening of drugs for anxiolytic potential in the FPT was published by Aron et al. (1971), based on an earlier report by their group (Boissier et al., 1968). In this screening study, broad groupings of drugs were tested, including representative psychotropics (6 drugs), hypnotics (6 drugs), anticonvulsants (8 drugs), antihistamines (5 drugs), neuroleptics (10 drugs), anti-parkinsonism drugs (4 drugs), analgesics (5 drugs), mild tranquilizers (9 drugs), and multiple grouping/hard to classify agents (10 drugs). While using the male Swiss mouse in such screening tests, the number of potential

doses employed was 11, covering a dose range of slightly greater than 1,000-fold. While this would have offered a robust potential dose testing scheme in terms of treatments used and dose range, it was never fully implemented for any single agent. The actual number of doses tested ranged from 2 to 9, with most falling into 4 to 6 doses tested. The dose range over which these drugs were assessed always fell far short of the maximum 1,000-fold range with most in the 4- to 32-fold zone, with only one dose range exceeding 32 (i.e., 300-fold dose range).

The screening results revealed that the entire set of nine minor tranquilizers increased the punished behavior, thereby showing the anxiolytic response (Figure 29). In general, these agents showed evidence of a biphasic dose response with the exception of meprobamate which was only tested with three doses. Consistent with the hormetic dose response, there was a ceiling effect such that most maximum responses did not exceed approximately twice the control value. Evaluation of the other pharmacological/drug treatment groups revealed occasional examples of anxiolytic acting agents (e.g., anti-parkinsonism: scopolamine, procyclidine; analgesics: dextropropoxyphene; antidepressants: amitriptyline, clomipramine; minor tranquilizers: fluoresone, hydroxyzine, tetrazepam), most of which also displayed hormetic-like dose-response relationships. Of particular note were the antihistamines, in which three of the five agents tested showed evidence of anxiolytic effects (Figure 30).

As a result of the initial extensive evaluation the FTP has enjoyed successful application for screening, exploring a wide range of receptor systems such as CCK_B ligands (Dooley and Klamt, 1993), SSRI (Hascoet et al., 2000), MAO inhibitors (Hascoet et al., 2000), oxytocin (Ring et al., 2006), and the 5-HT family of receptors (Dhonnchadha et al., 2003). As was

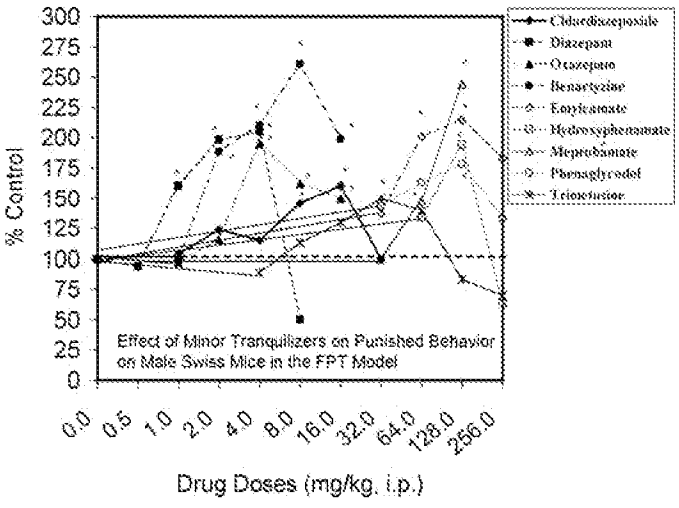

FIG. 29. Effect of minor tranquilizers on punished behavior on male Swiss mice in the FPT model. *Significantly different from controls at $p < .05$. (Source: Aron et al., 1971, Table 2, p. 463).

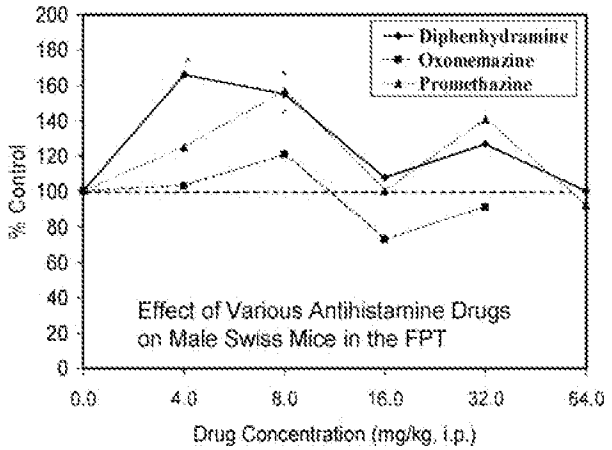

FIG. 30. Effect of various antihistamine drugs on punished behavior on male Swiss mice in the FPT model. *Significantly different from controls at $p < .05$. (Source: Aron et al., 1971, Table 3, p. 464).

the case with the initial screening tests of Aron et al. (1971), these studies have been more concerned with the elucidation of the anxiolytic effect rather than the assessment of the broader dose response relationship. Nonetheless, even though deemphasized, the hormetic-like biphasic dose response was commonly reported as seen with urotenion II (Do-Rego et al., 2005), oxytocin (Ring et al., 2006), and various antidepressants (Hascoet et al., 2000), among others.

In addition to the punished behavior aspects of the FPT, some investigators have employed the testing framework of the FPT without use of the electric shock in order to assess responses to a simple novel environment. Using male Albino mice (ICI), Marriott and Smith (1972) assessed the effects of six drugs on plate crossing in naive mice using four to seven doses, depending on the agent. In the cases of chlordiazepoxide, diazepam, meprobamate, and chlorpromazine, biphasic dose responses were observed in which low doses increased activity while high doses decreased activity (Figures 31–34). The maximum stimulatory response was modest in each case, with all drugs having a maximum response less than 150%. The width of the stimulatory response was also modest, ranging from 2- to slightly over 10-fold. Each of the responses was generally consistent with the hormetic dose-response relationship. Thus, regardless of whether shock was employed or not, the hormetic dose response was observed with similar quantitative features of the dose response.

SOCIAL INTERACTION TEST

In 1980 File created what has come to be called the social interaction test (SIT) to screen for possible anxiolytic drugs. At that time, all anxiety screening tests, except where locomotor activity was punished, used food or water deprivation to distinguish possible antianxiety effects of drugs from effects on

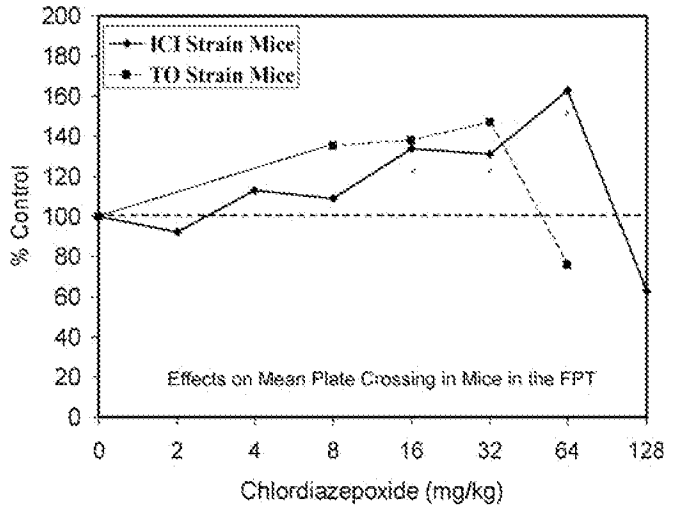

FIG. 31. Effects of chlordiazepoxide on plate-crossing in two different strains of mice naïve to the apparatus in the FPT model. *Significantly different from controls at $p < .05$. (Source: Marriott and Smith, 1972, Figure 2, p. 401).

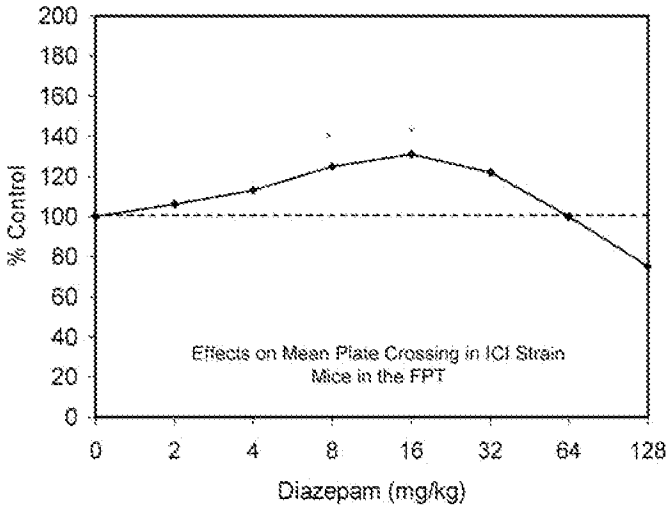

FIG. 33. Effects of diazepam on plate-crossing in ICI mice naive to the apparatus in the FPT model. *Significantly different from controls at $p < .05$. (Source: Marriott and Smith, 1972, Figure 2, p. 401).

motivation. She noted that a test of anxiety that does not involve deprivation, painful stimuli, or conditioned fear would be a valuable tool in drug screening. With such considerations the SIT of anxiety was created. In the SIT, uncertainty is created by placing rats in an unfamiliar setting in which the nature, location, and timing of noxious events are unknown and by manipulating the light intensity. Since rats have an unconditioned aversion for brightly lit areas, it was expected that manipulating light levels would cause changes in the rat's internal state that would be similar to those generated in humans by anxiogenic factors. According to File (1980), the behavior that is measured is the social interaction between pairs of rats and there is no need to deprive the animals or expose them to harmful stimuli such as shock.

The length of time that pairs of male rats spend in active social interaction is affected by the test conditions, being increased when rats are placed in a familiar area that is also under a low level of light intensity. When the lighting intensity is increased or the area is not familiar, the active social interaction is reduced. The decreased social interaction is not related to an increase in

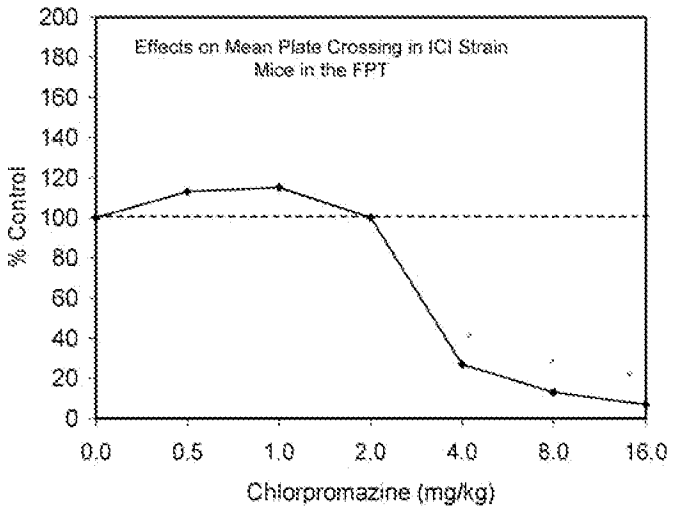

FIG. 32. Effects of chlorpromazine on plate-crossing in ICI mice naive to the apparatus in the FPT model. *Significantly different from controls at $p < .05$. (Source: Marriott and Smith, 1972, Figure 2, p. 401).

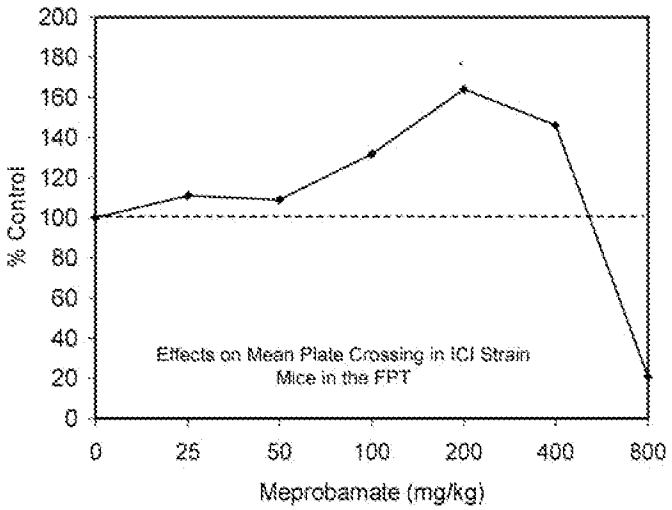

FIG. 34. Effects of meprobamate on plate-crossing in ICI mice naive to the apparatus in the FPT model. *Significantly different from controls at $p < .05$. (Source: Marriott and Smith, 1972, Figure 2, p. 401).

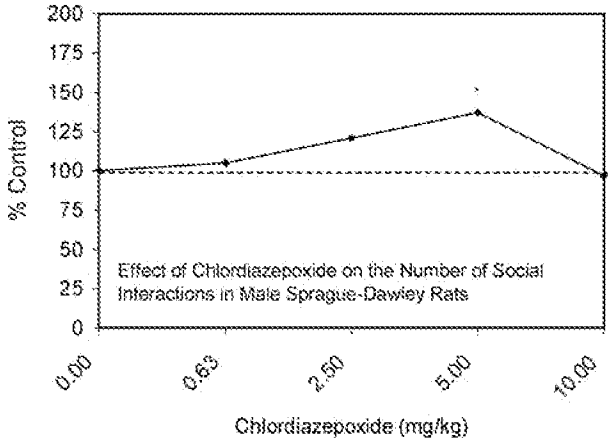

FIG. 35. Effect of chlordiazepoxide on the number of social interactions in male Sprague-Dawley rats. Significantly different from controls at $p < .05$. (Source: Millan et al., 2001, Figure 2, p. 590).

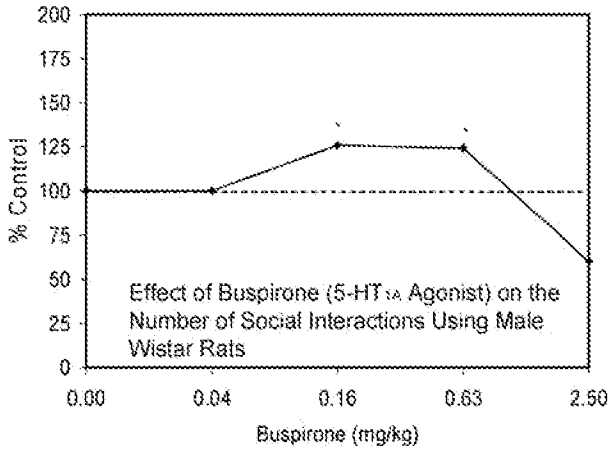

FIG. 37. Effect of buspirone (5-HT_{1A} agonist) on the number of social interactions using male Wistar rats. *Significantly different from controls at $p < .05$. (Source: Dekeyne et al., 2000, Figure 3, p. 60).

exploratory behavior when the animals are tested in an unfamiliar area, nor is it because of change in olfactory cues from the partner in the different test conditions.

The rats are selected on the basis of weight in order to ensure that a large rat is not evaluated with a smaller one, since this might change the nature of the interactions if one rat were clearly dominant over the other. The pairs of rats are evaluated over a 10-min duration using a video monitor. The behaviors that are scored and classified as active social interaction are sniffing, following, grooming, kicking, mounting, jumping on, wrestling and boxing with, and crawling under or over the partner. Passive body contact is scored separately as when the rats are sitting or lying with their bodies in contact, but without interacting with each other.

As has been the case with other tests of anxiety in animal models, the social interaction test (SIT) has routinely displayed hormetic-like biphasic dose-response relationships that have been independent of the chemical class, agent tested, and the animal model used. The present assessment demonstrates that such hormetic dose responses have occurred with drugs representing groups such as antidepressants (e.g., maprotiline, trazodone), benzodiazapines [diazepam, chlordiazepoxide (Figure 35)], NK_1 antagonists (e.g., NKP 608), 5-HT_{1A} agonists [e.g., S-20499 (Figure 36), S-20244, buspirone (Figure 37), 8-OH-DPAT, flesinoxan (Figure 38)], 5-HT_3 antagonist [e.g., GR38032F (Figure 39)], 5-HT_{2C} antagonists (e.g., SB242,084, SB206,553), noradrenaline reuptake inhibitor (e.g., MCI-225), and N_2O (Figure 40). These drugs have been tested with male

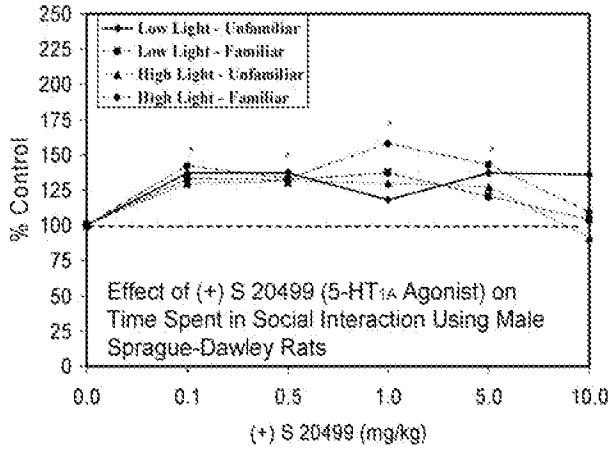

FIG. 36. Effect of (+) S 20499 (5-HT_{1A} agonist) on time spent in social interaction using male Sprague-Dawley rats. *Most treatments (18/24) were significantly different from controls at $p < .05$. (Source: Curle et al., 1994, Figure 5, p. 187).

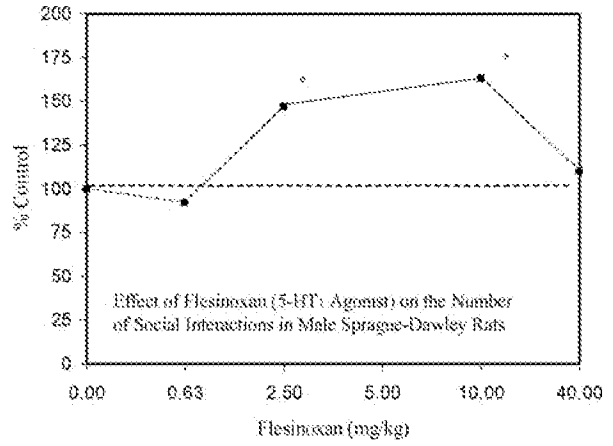

FIG. 38. Effect of flesinoxan (5-HT_1 agonist) on the number of social interactions in male Sprague-Dawley rats. *Significantly different from controls at $p < .05$. (Source: Millan et al., 2001, Figure 2, p. 590).

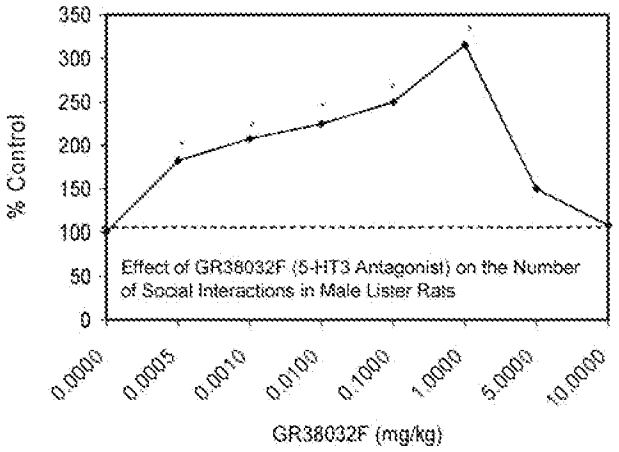

FIG. 39. Effect of GR38032F (5-HT_3 antagonist) on the number of social interactions in male Lister rats. *Significantly different from controls at $p < .05$. (Source: Jones et al., 1988, Figure 1, p. 988).

rats of four different strains, including the Sprague-Dawley, Wistar, Hooded-Lister, and Long-Evans, with the Sprague-Dawley strain being the most commonly employed, followed by the Wistar strain.

The dose-response characteristics displayed within the various strains have been consistent with those reported in other testing methods for anxiolytic drugs and with other hormetic endpoint responses that are independent of biological model or chemical class. The magnitude of the stimulation represented in these SIT tests is typically modest with an average maximum range of about 30–60% above the control response. Occasionally, some responses have approached being approximately twice that of the controls. These findings are consistent with a ceiling effect concept in pharmacology, which appears to define

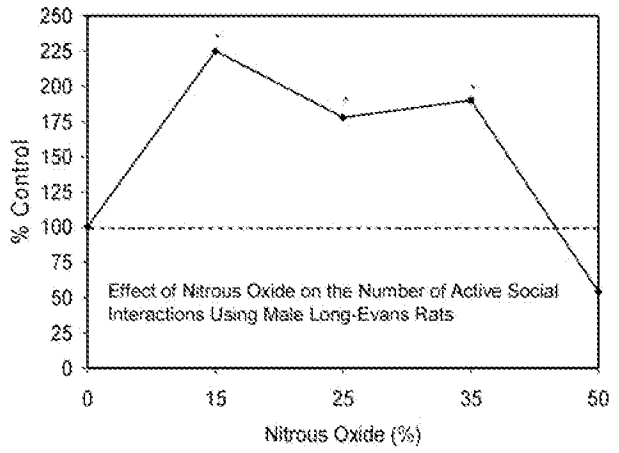

FIG. 40. Effect of nitrous oxide on the number of active social interactions using male Long-Evans rats. *Significantly different from controls at $p < .05$. (Source: Quock et al., 1993, Figure 2, p. 162).

a form of plasticity of the behavioral system under evaluation. The width of the stimulatory response has been more variable than the magnitude of the stimulatory response, reflecting possible heterogeneity within the animal model. In the case of the SIT about 80% of the examples have stimulatory widths less than 20-fold, although in the case of the NK_1 antagonist NKP608, the stimulatory width was approximately 1,000-fold, while in the case of the 5-HT_3 antagonist GR38032F, a remarkable range of about 50,000-fold was observed.

The SIT test has been reported in papers in which other screening tests of anxiolytic agents have been conducted with the same agents, thereby permitting intertest comparisons. For example, Jones et al. (1988) reported that the 5-HT_3 antagonist GR38032F biphasically affected social interaction with a low dose stimulation and a decline at higher doses. However, this drug failed to significantly affect the number of shocks received by the rats (Jones et al., 1988). In contrast to the negative response in the conflict–water lick test, this agent reduced anxiety in the light–dark exploration test with mice (Jones et al., 1988) with a dose response that was also biphasic with a maximum stimulation of about 80% and a stimulatory range of about 80-fold.

In addition to comparative evaluations across drug screening tests, models, drugs, and protocol variations, other investigations have clarified receptor pathways that may be involved in the anxiolytic response. For example, the effects of nitrous oxide and chlordiazepoxide in the SIT were reduced by flumazenil pretreatment, thereby indicating that nitrous oxide induces an anxiolytic response via benzodiazepine receptors (Quock et al., 1993). Similar mechanistic-oriented research was reported by Vassout et al. (2000), who linked the NK_1 antagonist NKP608 induced anxiolytic response to this receptor pathway via studies in the gerbil. In the case of GR38032F, the doses of this agent that blocked peripheral 5-HT_3 receptors in vivo without having any effects on 5-HT_{1A} and 5-HT_2 receptors in the rat are similar to those that are effective in the SIT (Jones et al., 1988).

While the biphasic dose response is often reported in the SIT, it is not often discussed in detail by the investigators. However, Vassout et al. (2000), following their work on NK_1 antagonists, offered this insightful perspective:

> A final comment needs to address the dose-response curves which had been seen here in both tests of anxiety. These tended to be bell-shaped, indicating that the positive effect seen at relatively low doses was fading out at higher doses. Although it is at present unknown what is causing this form of dose-response relation two points should be considered: (i) a non-linear dose-response relationship is not uncommon in biological testing and its occurrence has previously been seen with various classes of compounds and in various experimental paradigms (Araujo et al., 1998; Sakurada et al., 1999; Kopf et al., 1999; Revel et al., 1998). In some of cases, the attenuation of the effect size at high doses is caused by side-effects such as general motor stimulation or sedation. To the best of our knowledge such an argumentation cannot be used for NKP608, since our studies with up to very high doses of this compound have failed to detect any motor incapacitating effects up to 1000 mg/kg p.o. (unpublished data). (ii) interestingly enough, a comparable bell-shaped dose-response curve

with NKP608 has been recently observed in the chronic mild stress model in rats (Papp et al., 2000). It should be emphasized that bell-shaped dose-response curves are often only seen when a sufficiently broad dose range is included in a study. (p. 15)

VOGEL CONFLICT TEST

Conflicting choices are common in every day human activities. Such conflict-involved situations can become an important source of anxiety. An assessment of conflict situations and their clinical intervention has long been a major area of research in psychology. This is particularly the case with respect to the detection of anxiety-reducing (anxiolytic) agents. Numerous experimental protocols to detect anxiolytic agents have been developed and validated in animal models. Often such tests for anxiolytic agents are based on the natural behavior of the model, such as rodents, which fluctuate between a strong interest to investigate novel yet threatening environments, and the accompanying fear. Such conflict situations have included the assessment of spontaneous (untrained) behaviors and situations in which the subjects received punishment (e.g., mild electric shocks) that led to a suppression of a (learned) response for reinforcement (water/food) (Rodgers, 1997). In 1960, Geller and Seifter introduced a punishment-based conflict procedure to identify and characterize anxiolytic agents. In their procedure rats are trained to press a lever in order to receive a food or water reward. When the so-called "conflict" is presented (i.e., the "conflict" results from having the option of receiving an electric shock as a consequence of acceptings a food/water reward), responses (i.e., rewards) are inhibited by mild electric shocks. Anxiolytic properties are believed to be demonstrated when drugs selectively enhance the punished response.

In the Vogel conflict test (Vogel et al., 1971) male rats are water deprived for 48 h, and during a test period of 3 min, drinking water is punished by a mild but aversive shock delivered via the spout of the bottle once every 20 licks. According to theory, a specific, drug-induced increase in the number of shocks taken indicates anxiolytic properties. Over the subsequent years numerous alterations in the basic testing procedure have been introduced relating to duration of water or food deprivation, length of session, shock intensity/frequency, and animal model (e.g., mouse rather than rat).

A number of researchers have explored the effects of a broad dose range during the Vogel or related conflict tests (Hjorth et al., 1986; Sonderpalm and Engel, 1988; Engel et al., 1989; Dekeyne et al., 2000; Deren-Wesolek et al., 1998; Umezu, 1999; Mathiasen and Mirza, 2005; Vaidya et al., 2005; Wiley et al., 1998). Some of the drugs tested have included apomorphine (Figure 41) (Hjorth et al., 1986), clonidine (Figure 42) (Sonderpalm and Engel, 1988), buspirone (Figure 43) (Vaidya et al., 2005), amperozide (Figure 44) (Engel et al., 1989), diazepam (Figure 45) (Wiley et al., 1998), and L-5-HTP (Figure 46) (Hjorth et al., 1987). They have been tested in a variety of animal models including male Sprague-Dawley, Wistar, and Long-Evans rats and the C57/B mouse. The modes of action have been reported to

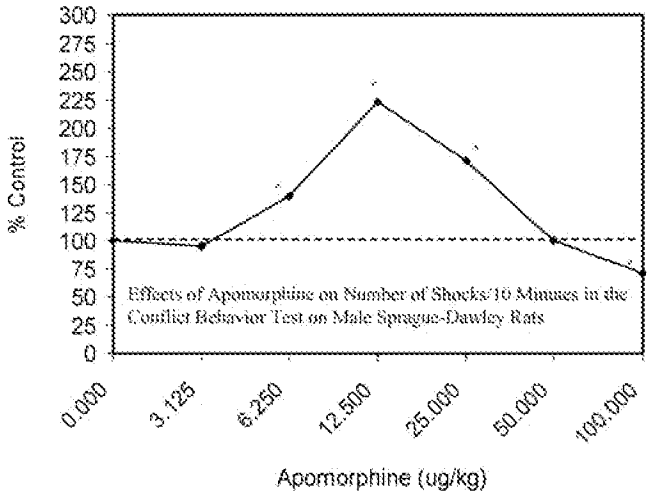

FIG. 41. Effects of apomorphine on conflict behavior in male Sprague-Dawley rats. *Significantly different from controls at $p < .05$. (Source: Hjorth et al., 1986, Figure 1, p. 238).

be quite diverse, involving several receptor pathways, including α-adrenergic, benzodiazepine, dopamine, and 5-HT. In the case of amperozide, the stimulatory response was blocked by administering the BDZ receptor antagonist bicuculline (Engel et al., 1989). Despite the use of different animal models, varied protocols, and different modes of action, the dose responses may be characterized as being hormetic-like biphasic dose-response relationships with similar quantitative features. As can be seen in Figures 41–46, the stimulatory response was generally modest with a stimulatory concentration range being about 4- to 30-fold.

It is interesting to note that in several of the already cited papers showing a biphasic dose response in the Vogel conflict

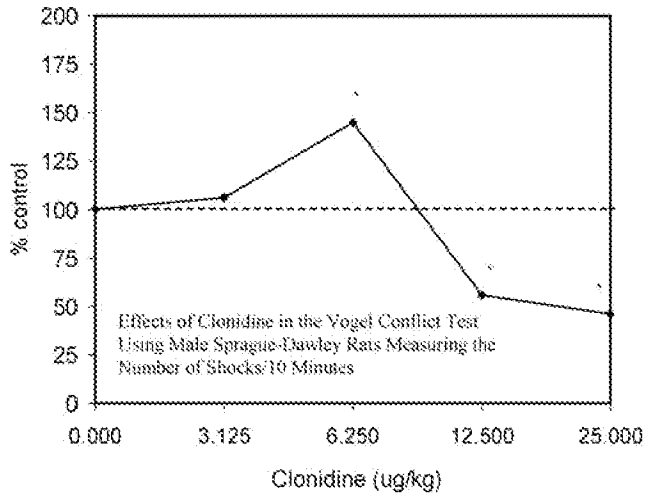

FIG. 42. Effects of clonidine in the Vogel conflict test using male Sprague-Dawley rats measuring the number of shocks/10 min. *Significantly different from controls at $p < .001$. (Soderpalm and Engel, 1988, Figure 1, p. 473).

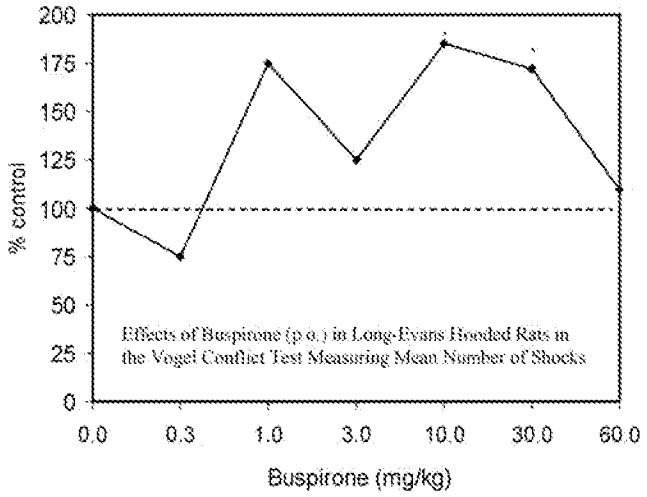

FIG. 43. Effects of buspirone (p.o.) in Long-Evans hooded rats in the Vogel conflict test measuring mean number of shocks. *Significantly different from controls at $p < .05$. (Source: Vaidya et al., 2005, Figure 1, p. 249).

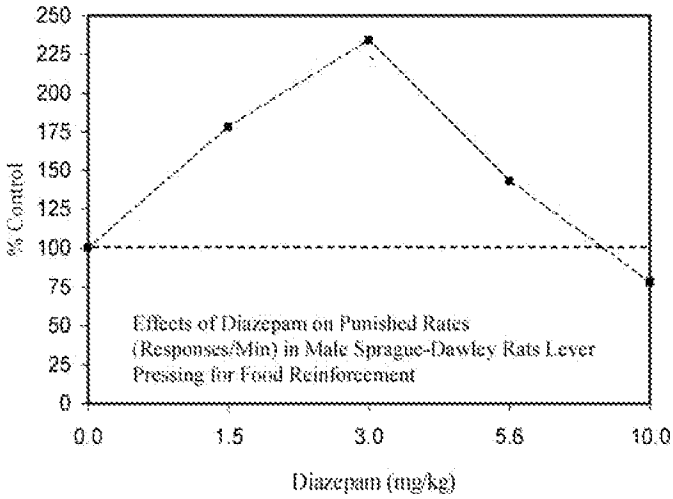

FIG. 45. Effects of diazepam on punished rate (responses/min) in male Sprague-Dawley rats lever pressing for food reinforcement. *Significantly different from controls at $p < .05$. (Source: Wiley et al., 1998, Figure 1, p. 1530).

test, the elevated maze test (Figures 47–49) was also performed. The hormetic-like biphasic dose response was observed in both testing protocols. However, these testing protocols (Vogel vs. EPM) employed different study designs in terms of the dose range selected and time period between dose administered and evaluation. While it is clear that the hormetic response occurs for both endpoints, it also appears likely that the Vogel conflict and EPM test endpoints have variable quantitative characteristics and may involve different underlying mechanisms.

STAIRCASE TEST

The staircase test was developed by Thiebot et al. (1973, 1976) based upon a procedure initially proposed by Molinengo and Ricci-Gamalero (1970). In the initial formulation of this test, a rat was placed within an enclosed staircase and the numbers of steps climbed (e.g., stairs could be climbed more than once) and rearing responses were recorded. In the 1980s Simiand et al. (1984) adapted the staircase test for use with mice. Since that time, the use of the mouse model in this test has become predominant in the published literature. Upon placement of naive animals into the enclosed staircase, initially a negative and then

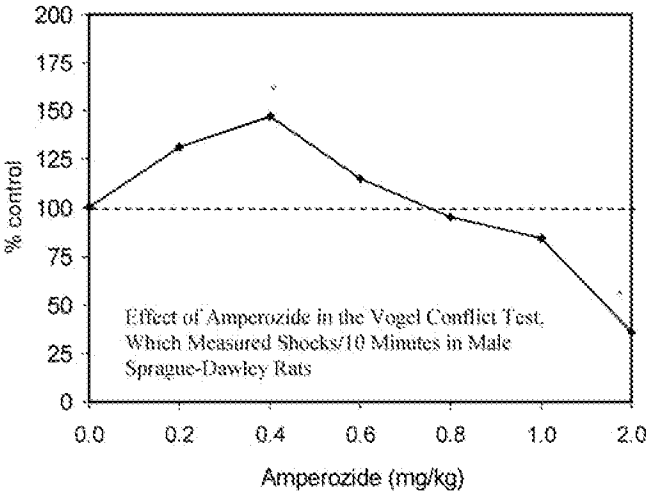

FIG. 44. Effect of amperozide in the Vogel conflict test, which measured shocks/10 min in male Sprague-Dawley rats. *Significantly different from controls at $p < .001$. (Source: Engel et al., 1989, Figure 1, p. 430).

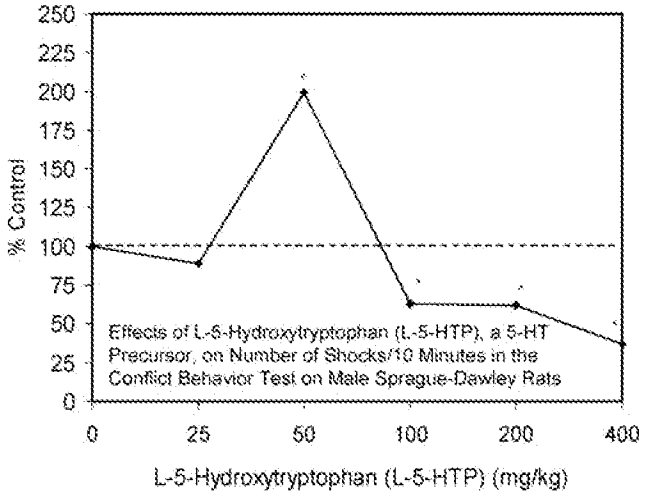

FIG. 46. Effect of L-5-hydroxytryptophan (L-5-HTP), a 5-HT precursor, on number of shocks/10 min in conflict behavior in male Sprague-Dawley rats. *Significantly different from controls at $p < .05$. (Source: Hjorth et al., 1987, Figure 1, p. 97).

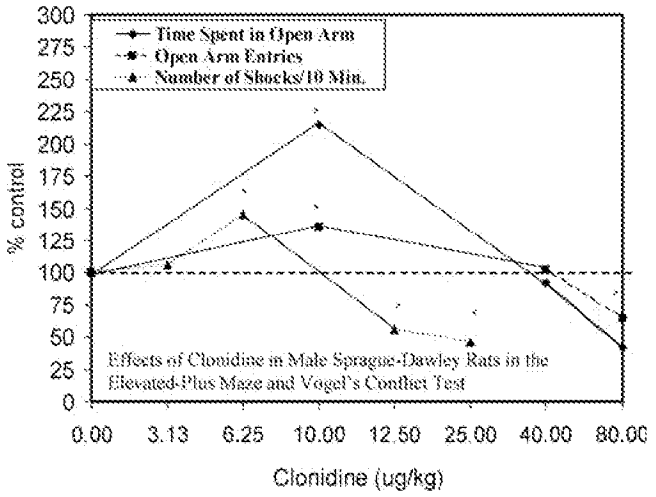

FIG. 47. Effects of clonidine in male Sprague-Dawley rats in the elevated-plus maze (i.e., Montgomery's conflict test) and Vogel's conflict test. *Significantly different from controls at $p < .001$. (Source: Soderpalm and Engle, 1988, Figures 1 and 2, pp. 473 and 474).

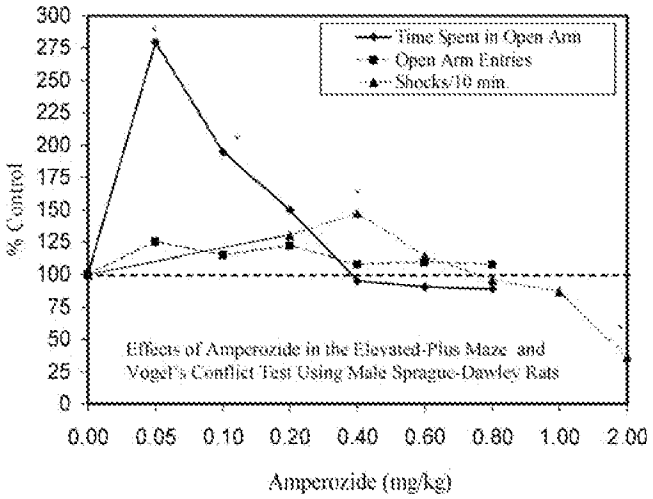

FIG. 49. Effects of amperozide in the elevated-plus maze (i.e., Montgomery's conflict test) and Vogel's conflict test using male Sprague-Dawley rats. *Significantly different from controls at $p < .05$. (Source: Engel et al., 1989, Figures 1 and 31, pp. 430 and 432).

a positive relationship between the steps climbed and the rearing was observed. These observations led Thiebot et al. (1973, 1976) to suggest that the rearings reflected emotionality or anxiety while the step climbing was considered as an index of exploration (Boissier et al., 1976). These interpretations had their origins in the earlier work of Lat (1965), who suggested that the upward rearings offered an index of a specific state of awareness or vigilance. Later work by Soubrie et al. (1977) revealed that rearings were directly related to locomotion rather than exploration. Using the open-field test they reported that during the initial minutes of the testing procedure, the locomotor response is an expression of anxiety in which escape activity predominates and only later does the exploratory component occur. Since the rearing behavior was associated with escape exploration they suggested that during the first few minutes of the test the rearing response was an aspect of anxiety.

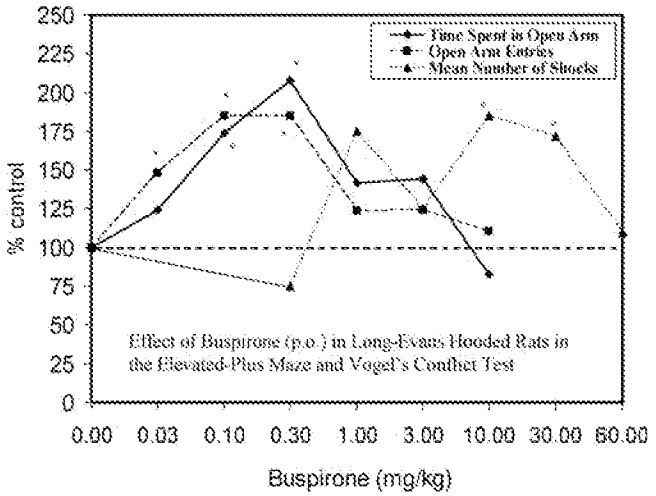

FIG. 48. Effect of buspirone (p.o.) in Long-Evans hooded rats in the elevated-plus maze (i.e., Montgomery's conflict test) and Vogel's conflict test. *Significantly different from controls at $p < .05$. (Source: Vaidya et al., 2005, Figures 1 and 3, pp. 249 and 250).

In practice, anxiolytic agents would be expected to cause a decrease in rearing activity while either stimulating or having no effect on stair climbing. The actual stair climbing itself is considered to be more related to exploratory or locomotor activity.

Despite these assumptions and interpretations, Simiand et al. (1984) argued that even though rearing and locomotion can be decoupled by anxiolytic and psychotropic drugs (see Hughes, 1972; Cunha and Masur, 1978), both behaviors probably have a significant exploratory component. They argued further that the precise relationship of rearing to anxiety and climbing to exploration remains to be clarified. While theoretical uncertainties remain to be resolved, it is generally accepted that the staircase test can be a valuable means to screen for anxiolytic drugs.

The staircase test has important practical features since it is simple, quickly conducted, economical, and reasonably selective for anxiolytic drugs. Furthermore, it does not demand prior handling of the animals, nor does it involve training to various performance levels, food deprivation, or habituation, all of which can be limitations in other anxiolytic testing protocols.

Dose-Response Relationships

In the staircase test, steps taken and rearings are counted over a 3-min period and then compared. No data have been presented in which these two endpoints were assessed over different time

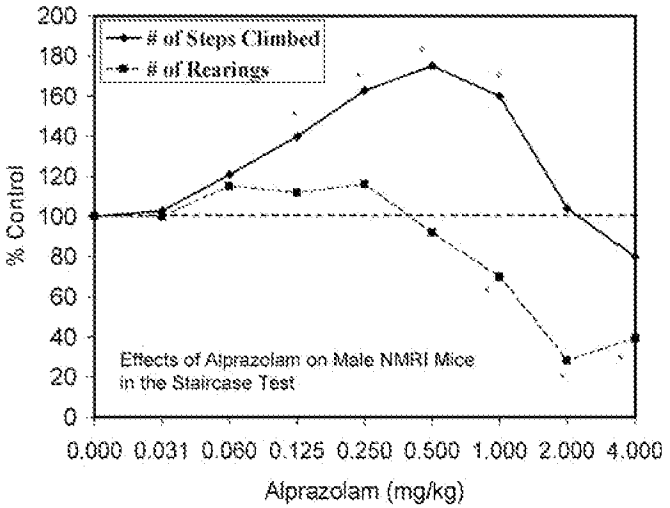

FIG. 50. Effects of alprazolam on the number of steps climbed and the number of rears in the staircase test in male NMRI mice. *Significantly different from controls at $p < .05$. (Source: Steru et al., 1987, Figure 1, p. 107).

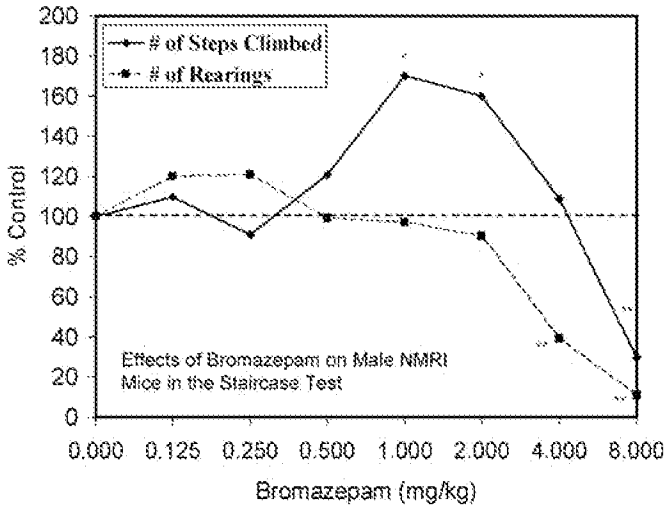

FIG. 51. Effects of bromazepam on the number of steps climbed and the number of rears in the staircase test in male NMRI mice. *Significantly different from controls at $p < .05$. (Source: Steru et al., 1987, Figure 1, p. 107).

points (e.g., 3 min for steps and 5 min of rearings or other arrangements). There has also been no set study design guidance features for assessing the dose response, as some studies have included as few as 3 doses while others have used up to 14 doses. Nonetheless, a set of studies with robust designs (i.e., number and range of doses) exists for evaluation.

Essentially all studies dealing with dose responses are assessed with respect to whether the drug reduces rearings. If there is a dose-dependent decrease the agent is deemed to have anxiolytic properties. The response is then assessed for whether these doses also induced exploratory behavior. However, Steru et al. (1987) have revealed that benzodiazepines that have been tested in detailed dose-response studies have generally shown biphasic dose responses for both steps climbed and rearing behavior (Figures 50–53). Two other agents that did not show the low dose stimulation in this study were not tested below the threshold. The quantitative features of the exploratory response had a higher maximum stimulation and a broader stimulatory range than the rearing behavior, although both endpoints were consistent with the quantitative features of the hormetic dose response. Similar dose responses were also reported for ethanol, phencyclidine, and nicotine (Figures 54 and 55) (Pollard and Howard, 1986). Boissier et al. (1976) observed a nonparallel variation between the climbing and rearing parameters with meprobamate and amobarbital in rats (Figure 56, a and b). Similar nonparallel responses were also reported by Simiand et al. (1984) for phenobarbital and meprobamate in the mouse model (Figure 57, a and b).

The biphasic nature of the dose response for the locomotor effect has been interpreted as most likely due to a receptor-mediated stimulation at low doses and a sedative effect at higher doses. While this seemed to be a likely explanation, several papers by Pick and colleagues have demonstrated that the benzodiazepine antagonist flumazenil prevented both the low-dose stimulation and high-dose inhibition by alprazolam, thereby indicating a GABA-mediated receptor for these opposing responses. These findings and their specificity challenge the sedation–high-dose inhibition hypothesis (Pick et al., 1996, 1997; Milman et al., 2006).

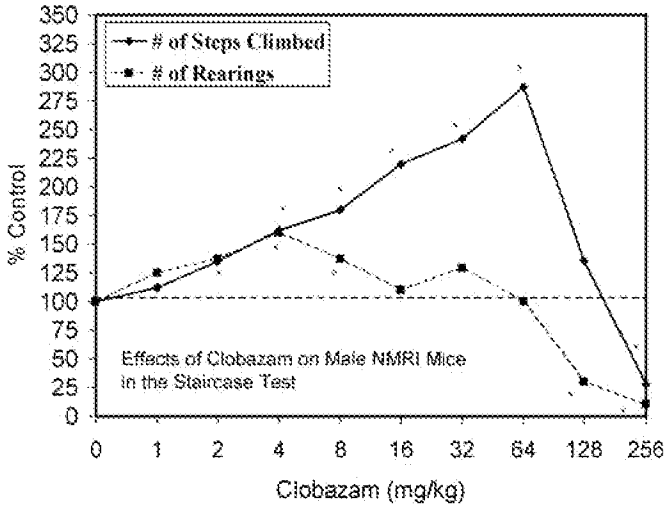

FIG. 52. Effects of clobazam on the number of steps climbed and the number of rears in the staircase test in male NMRI mice. *Significantly different from controls at $p < .05$. (Source: Steru et al., 1987, Figure 1, p. 107).

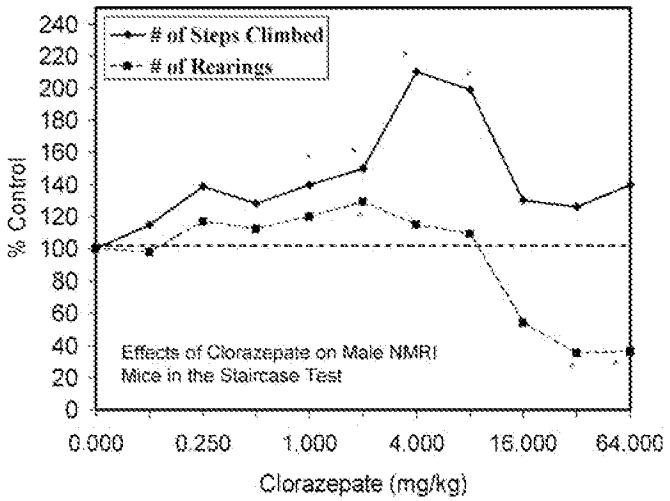

FIG. 53. Effects of clorazepate on the number of steps climbed and the number of rears in the staircase test in male NMRI mice. *Significantly different from controls at $p < .05$. (Source: Steru et al., 1987, Figure 1, p. 107).

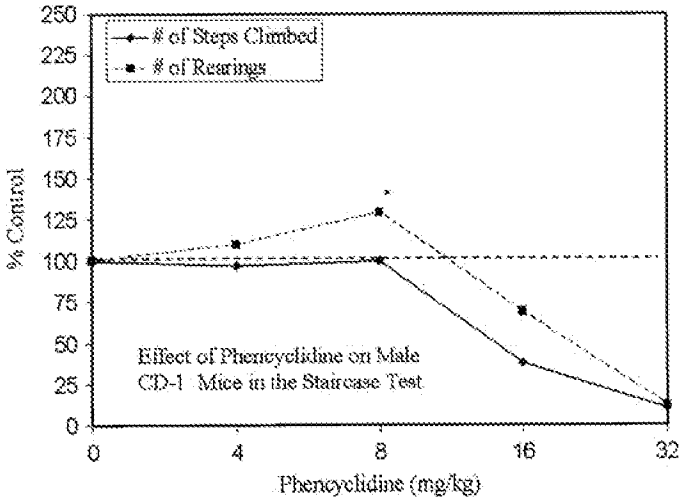

FIG. 55a. Effects of phencyclidine on male CD-1 mice in the staircase test. *Significantly different from controls at $p < .05$. (Source: Pollard and Howard, 1986, Figure 3, p. 16).

FREEZING BEHAVIOR/FEAR CONDITIONING

Serotonin-Dopamine Antagonist

Conditioned fear stress (CFS) occurs when an animal is placed in an environmental setting that had been previously linked or associated with an unpleasant experience such as a foot shock. Under such circumstances an animal model, such as a rat, displays very obvious freezing behavior (Fanselow, 1980). CFS is a type of psychological stress without physical stimuli and therefore has been of interest to researchers studying animal models with respect to anxiety, depression, or fear. Such research interests led to the development of the so-called CSF-induced freezing behavior test as an initial screen for certain types of anxiolytic agents (Inoue et al., 1992; Conti et al., 1990; Fanselow and Helmstetter, 1988) and antidepressants (Hashimoto et al., 1996; Beck and Fibiger, 1995).

The freezing behavior test involves a comparison of conditioned and nonconditioned animals. On the training day, the conditioned group of animals is placed (one animal at a time) into the experimental chamber with a grid floor and administered an unconditional stimulus (i.e., electric shock for 30 min). The nonconditioned group is similarly handled but without the electric shock. On the testing day, which occurs 1 day after the

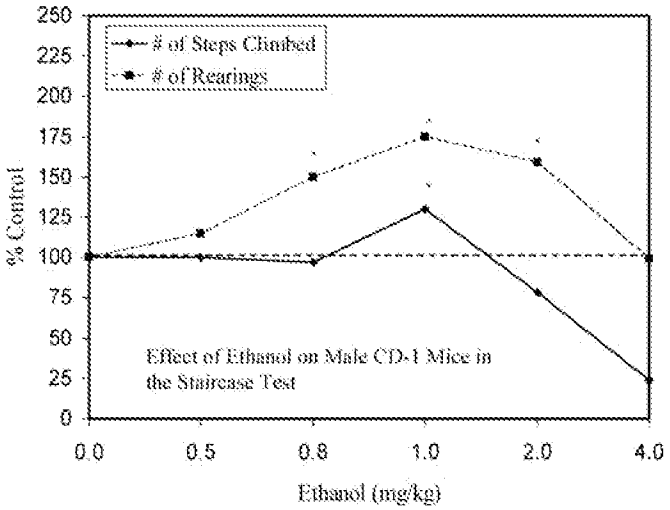

FIG. 54. Effects of ethanol on rearings and steps climbed in the staircase test in male CD-1 mice. *Significantly different from controls at $p < .05$. (Source: Pollard and Howard, 1986, Figure 1, p. 16).

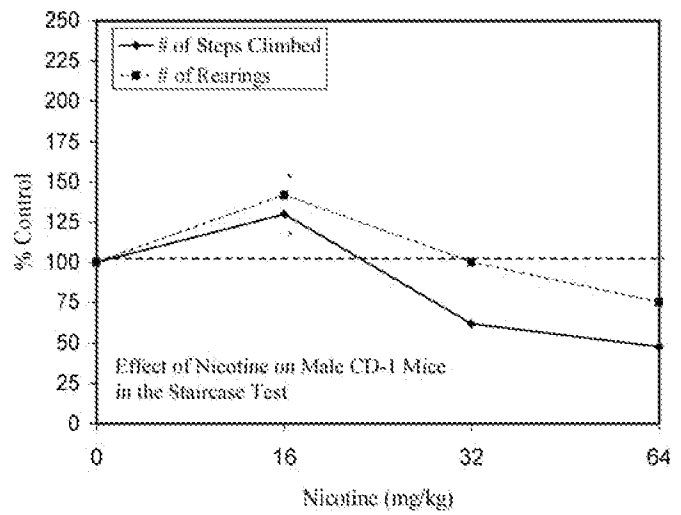

FIG. 55b. Effects of nicotine on male CD-1 mice in the staircase test. *Significantly different from controls at $p < .05$. (Source: Pollard and Howard, 1986, Figure 3, p. 16).

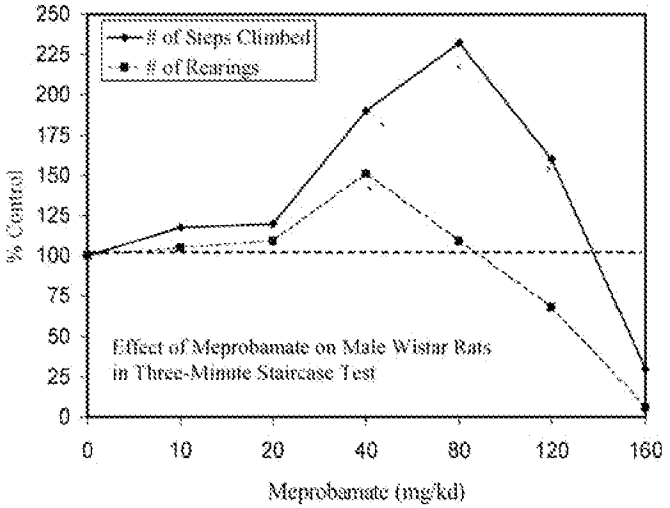

FIG. 56a. Effect of meprobamate on male Wistar rats in three minute staircase test. *Significantly different from controls at $p < .05$. (Source: Boissier et al., 1976, Figure 1, p. 215).

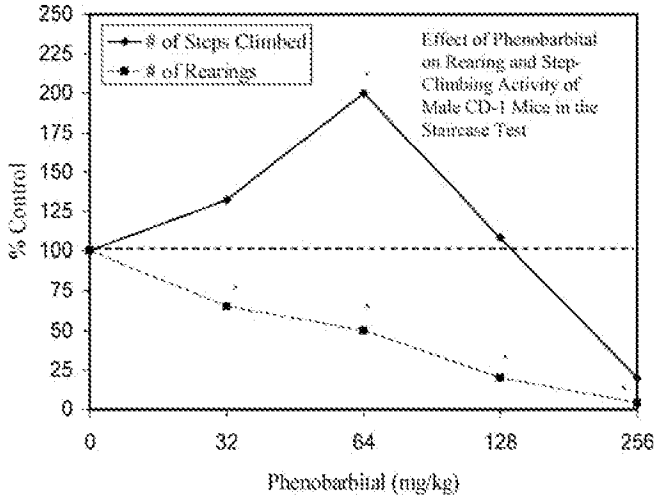

FIG. 57a. Effects of phenobarbital and meprobamate on rearing and step-climbing activity of male CD-1 mice in the staircase test. *Significantly different from controls at $p < .05$. (Source: Simiand et al., 1984, Figure 3, p. 51).

training session, the rats are returned to the chamber. The CFS-induced freezing behavior is measured for 5 min in the absence of the unconditioned stimulus (US). Each rat is rated as being either frozen or active every 10 s in a total of 30 sampling periods. Freezing behavior is defined as the complete lack of all observable movements of the body and vibrissae (nasal hairs) except those occurring due to respiration. To get a "freezing" score for a 10-s period the animal must be "frozen" for the entire 10 s. The score is the number of 10-s freezing sessions over the total of 30.

The freezing test has been used to evaluate the effects of perospirone, a novel serotonin-2 (5-HT_2) and dopamine-2 (D_2) receptor antagonist (SDA), on CFS-induced freezing behavior in male Sprague-Dawley rats for comparison to other antipsychotics (i.e., haloperidol, clozapine, risperidone, diazepam, mosapramine, thioridazine, tiapride, chlorpromazine, imipramine, desipramine, ketanserin, and ritanserin). In the test procedures the unconditioned rats were "freezing" roughly five to eight 10-s periods during the 30 sessions. In contrast, the conditioned rats were "frozen" between 20 and 25 times within the 30 sessions. In all cases the antipsychotic agents decreased the CFS-induced freezing behavior. Of these 13 drugs, 9 displayed evidence of a U-shaped dose response (Ishida-Tokuda et al., 1996) (Figures 58–67).

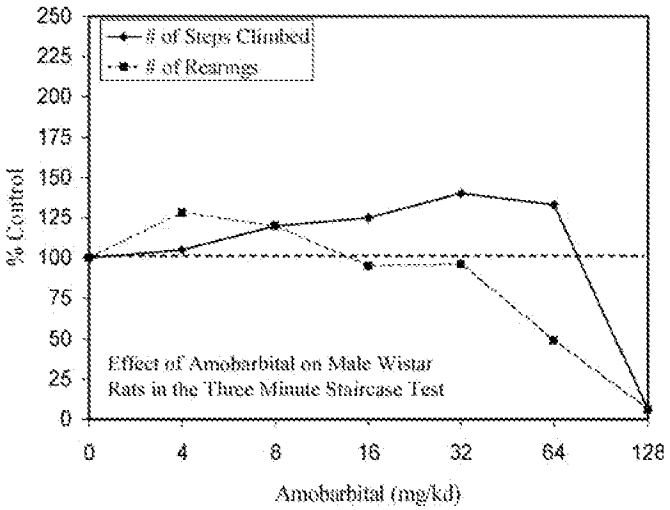

FIG. 56b. Effect of amobarbital on male Wistar rats in three minute staircase test. (Source: Boissier et al., 1976, Figure 1, p. 215).

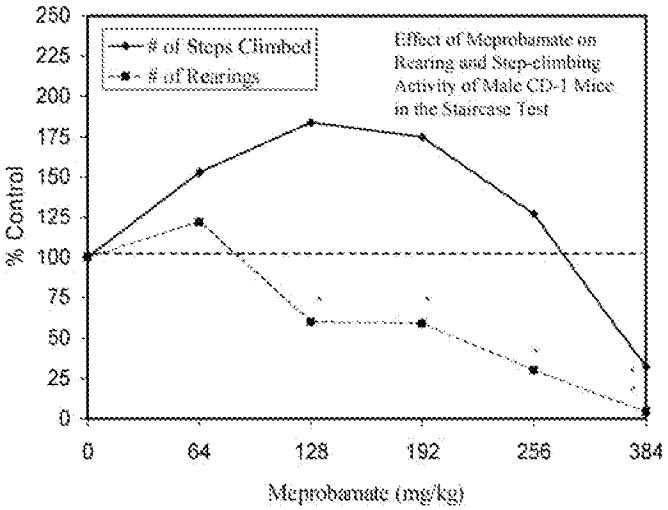

FIG. 57b. Effect of meprobamate on rearing and step-climbing activity of male CD-1 mice in the staircase test. (Source: Simiand et al., 1984, Figure 3, p. 51).

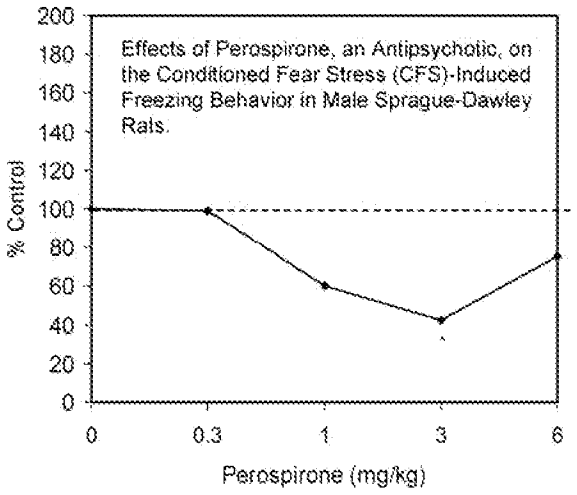

FIG. 58. Effects of perospirone, an antipsychotic, on the conditioned fear stress (CFS)-inducing freezing behavior in male Sprague-Dawley rats. *Significantly different from controls at $p < .05$. (Source: Ishida-Tokuda et al., 1996, p. 121, Figure 1).

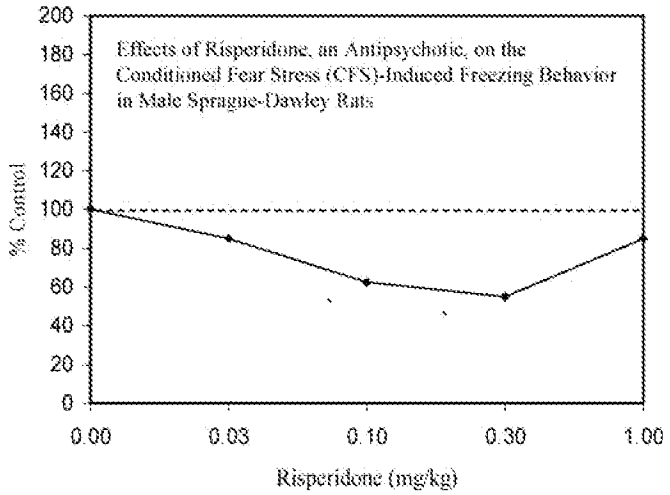

FIG. 60. Effects of risperidone, an antipsychotic, on the conditioned fear stress (CFS)-induced freezing behavior in male Sprague-Dawley rats. *Significantly different from controls at $p < .05$. (Source: Ishida-Tokuda et al., 1996, p. 121, Figure 2).

While the number of doses was not adequate to provide a detailed characterization of the dose response, the general body of evidence indicates that the maximum decrease in the occurrence of freezing ranged between 20 and 60%. The concentration range over which the decrease in freezing occurred was variable and, for the most part, not quantitatively clarified. However, it is obvious that roughly half of the drugs displayed a response range greater than 10-fold. Although they were unable to account for these findings, the authors argued that only SDA-type antipsychotics with combined 5-HT_2 and D_2 blocking actions reduced the occurrence of CSF-induced freezing behavior; this led to the conclusion that conventional antipsychotics such as haloperidol, chlorpromazine, thioridazine, and mosapramine did not reduce the CFS-induced freezing behavior. The present analysis concurs only with the judgment for chlorpromazine. The apparent U-shaped dose response for the other three drugs, while not as large and broad as for the other drugs, should not be dismissed without further evaluation.

The research of Ishida-Tokuda et al. (1996) was motivated by observations that schizophrenic patients display diverse symptoms with both "positive" (e.g., hallucination, delusion, and excitation) and/or "negative" (e.g., apathy, social withdrawal)

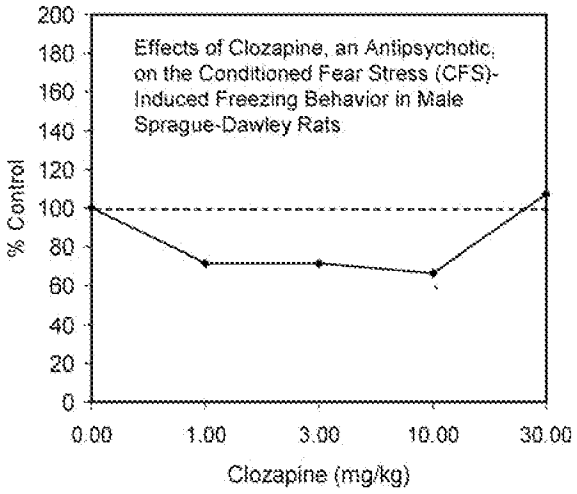

FIG. 59. Effects of clozapine, an antipsychotic, on the conditioned fear stress (CFS)-inducing freezing behavior in male Sprague-Dawley rats. *Significantly different from controls at $p < .05$. (Source: Ishida-Tokuda et al., 1996, p. 121, Figure 2).

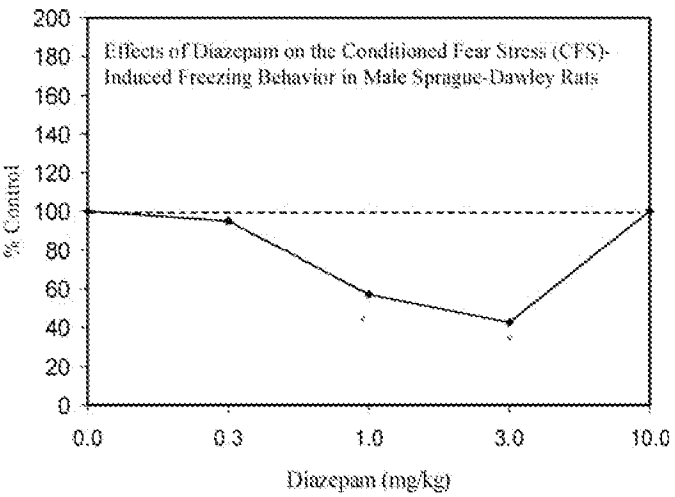

FIG. 61. Effects of diazepam on the conditioned fear stress (CFS)-induced freezing behavior in male Sprague-Dawley rats. *Significantly different from controls at $p < .05$. (Source: Ishida-Tokuda et al., 1996, p. 122, Figure 3).

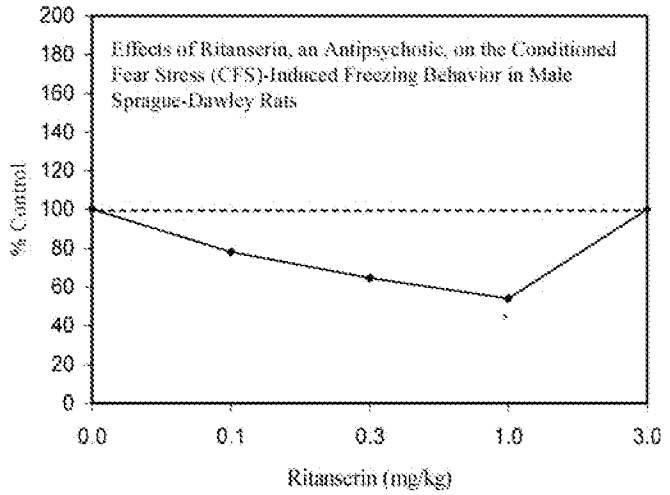

FIG. 62. Effects of ritanserin, an antipsychotic, on the conditioned fear stress (CFS)-induced freezing behavior in male Sprague-Dawley rats. *Significantly different from controls at $p < .05$. (Source: Ishida-Tokuda et al., 1996, p. 123, Figure 4).

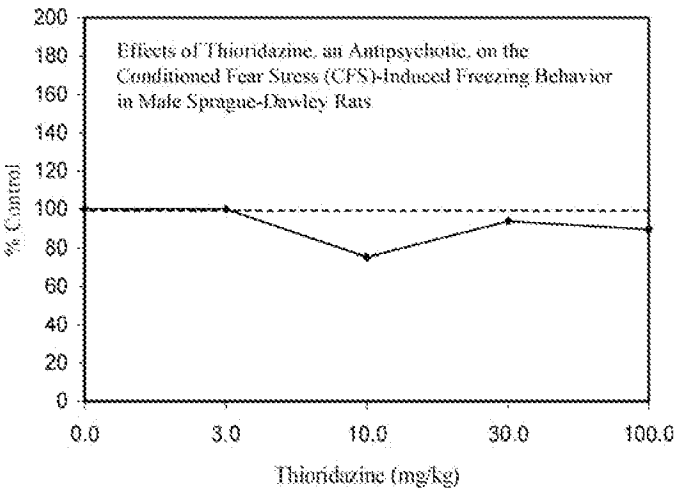

FIG. 64. Effects of thioridazine, an antipsychotic, on the conditioned fear stress (CFS)-induced freezing behavior in male Sprague-Dawley rats. (Source: Ishida-Tokuda et al., 1996, p. 122, Table 1).

symptoms and dysphoric mood alteration (e.g., anxiety and depression). The authors argued that the "positive" symptoms were effectively dealt with by conventional antipsychotics but not the so-called "negative" symptoms. However, a new class of drugs possessing both 5-HT_2 and D_2 actions had been reported to reduce such negative symptoms. While this was the underlying theoretical framework of the experiment, the findings indicated that for three of the four conventional antispychotics there is evidence of a U-shaped dose response with improvements in the negative symptoms. While the magnitude of the improvement appears less than the 5-HT_2 and D_2 blocking agents, their potential U-shape in the freezing behavior assessment needs to be confirmed.

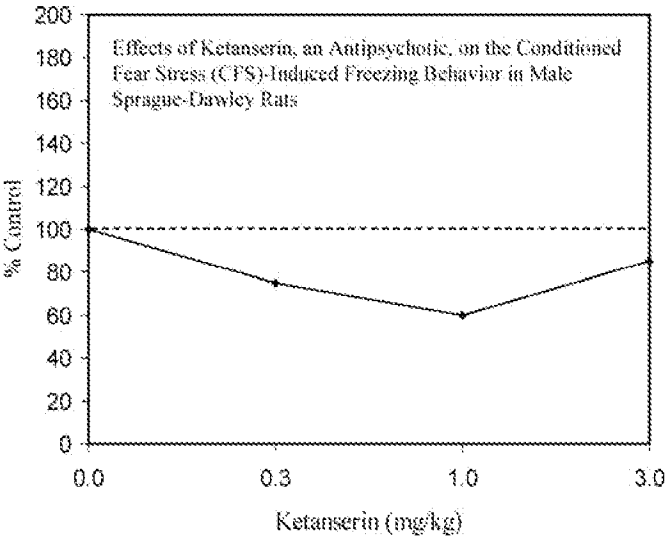

FIG. 63. Effects of ketanserin, an antipsychotic, on the conditioned fear stress (CFS)-induced freezing behavior in male Sprague-Dawley rats. (Source: Ishida-Tokuda et al., 1996, p. 123, Figure 4).

NMDA Receptor Modulation by Spermidine

The molecular foundations of pavlovian conditioning have been an area of considerable research interest. In the case of so-called classical fear conditioning, it is well recognized that the experimental model will acquire the ability to show a defensive response(s) to a neutral conditioned stimulus following an association with a noxious unconditional stimulus. The amygdala

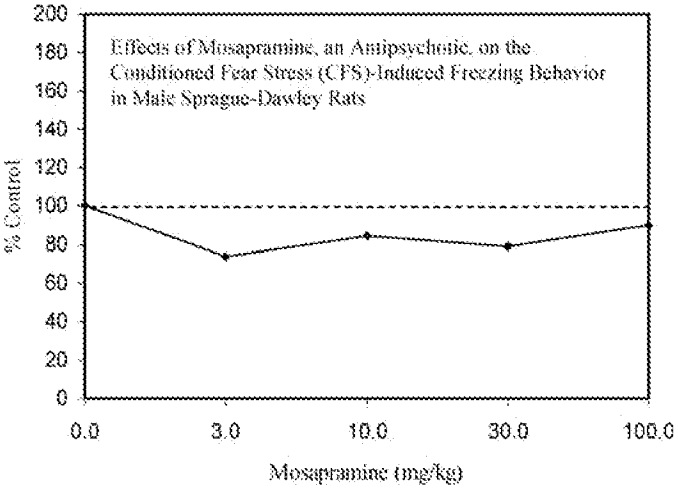

FIG. 65. Effects of mosapramine, an antipsychotic, on the conditioned fear stress (CFS)-induced freezing behavior in male Sprague-Dawley rats. (Source: Ishida-Tokuda et al., 1996, p. 122, Table 1).

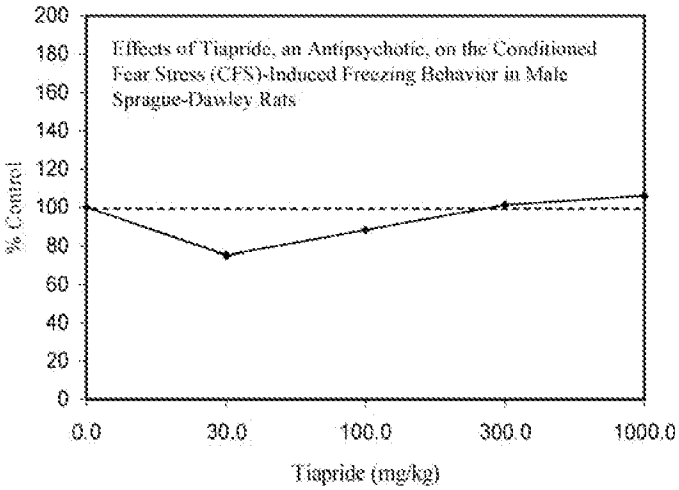

FIG. 66. Effects of tiapride, an antipsychotic, on the conditioned fear stress (CFS)-induced freezing behavior in male Sprague-Dawley rats. (Source: Ishida-Tokuda et al., 1996, p. 122, Table 1).

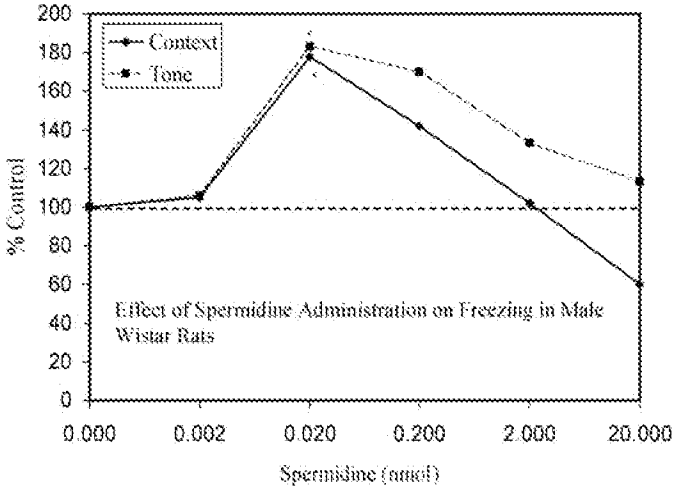

FIG. 68. Effect of pretraining intra-amygdala spermidine administration on freezing as measured to context and to tone in male Wistar rats. *Significantly different from controls at $p < .05$. (Source: Rubin et al., 2004, Figures A and B, p. 2330).

and its *N*-methyl-D-aspartate (NDMA) receptors have been associated with the acquisition and expression of fear conditioning (Kim et al., 1993; Maren et al., 1996; Goosens and Maren, 2001). In order to better understand how NMDA receptor functioning is modulated, particular attention has focused on the general class of polyamines (Williams et al., 1991; Rock and Macdonald, 1995). Various studies have revealed that polyamines biphasically affect memory consolidation and retrieval, with high doses (>125 nmol icv) decreasing and low doses (0.02–40 nmol) enhancing performance (Rubin et al., 2000, 2001). Since these studies were limited to the consolidation phase of the inhibitory avoidance test, Rubin et al. (2004) assessed whether intra-amygdala injections of polyamines affect the acquisition and consolidation of the fear conditioning response.

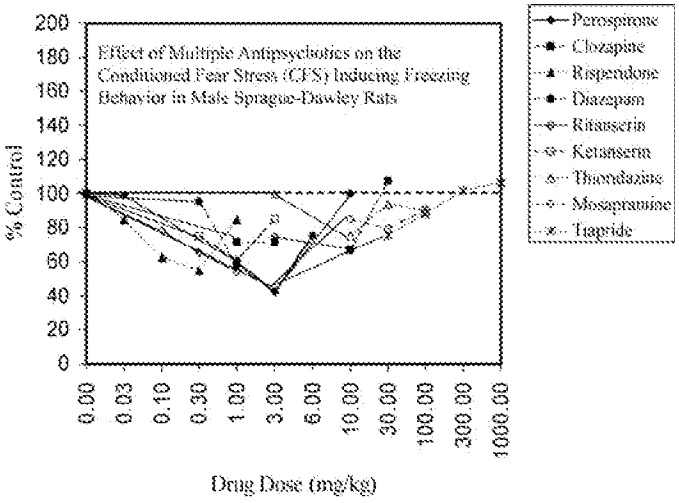

FIG. 67. Effect of multiple antipsychotics on the conditioned fear stress (CFS) induced freezing behavior in male Sprague-Dawley rats. (Source: Ishida-Tokuda et al., 1996, summarized Figures 58–66 of this article).

In their study adult male Wistar rats were trained to associate an auditory stimulus with a painful electric shock (i.e., fear conditioning) (Rubin et al., 2004). The measurement of the fear response was on the occurrence of "freezing," that is, lack of movement (excluding breathing). The polyamine spermidine was injected into the intra-amygdala either immediately pretraining or posttraining to assess modulation of the acquisition of contextual and auditory fear conditioning. The pre- or immediate posttraining mode of administration was critical in order to differentiate the acquisition from the early consolidation of fear conditioning. The experiment also included the impact of spermidine on the freezing response in a novel context without tone (i.e., contextual evaluation). The effects of pretreatment with spermidine affected both contextual and tone-conditioned fear responses in a biphasic manner, with a low-dose stimulation and a high-dose inhibition (Figure 68). The maximum stimulatory response was about 80% greater than the control response in both cases. The stimulatory responses were between several hundredfold and 1,000-fold concentration range for both contextual and tonal evaluations.

The posttraining administration of spermidine also enhanced freezing to the contextual auditory conditioned response in a biphasic dose-response manner, with the magnitude of enhanced response approaching about 80% greater than control responses. The administration of the NMDA antagonist arcaine decreased the freezing response. Likewise, arcaine coadministration reversed this effect of spermidine, even at doses at which arcaine had no effect by itself. At the doses where spermidine enhanced the conditioning response it had no effect on shock threshold,

anxiety, or locomotor ability, thereby eliminating motivational disparities on training as a factor accounting for the spermidine effects on the fear conditioning response.

Rubin et al. (2004) suggested that low doses of polyamines regulate amygdala function via the modulation of NMDA receptors. This interpretation is consistent with other findings of NMDA receptor modulation based on neurochemical and electrophysiological evidence (Williams et al., 1991; Rock and Macdonald, 1995; Williams, 1997). That is, polyamines biphasically affect MK-801 and TCP binding to the NMDA receptor channel (Williams et al., 1991; Rock and Macdonald, 1995; Williams, 1997) in similar fashion, and they biphasically effect NMDA-evoked currents (McGurk et al., 1990; Sprosen and Woodruff, 1990; Williams et al., 1990; Rock and Macdonald, 1995) with dose-response features quantitatively similar to those reported in the fear conditioning experiments of Rubin et al. (2004).

DEPRESSION BEHAVIOR (FORCED SWIM TEST, TAIL SUSPENSION TEST, AND CHRONIC STRESS TEST)

Antidepressant drug efficacy has been assessed by several behavioral tests in rodents, referred to as the forced swim test (FST), the tail suspension test (TST) (Bai et al., 2001; Bourin et al., 2005; Petit-Demouliere et al., 2005; Steru et al., 1985; Ripoll et al., 2003), and the unpredictable chronic stress test (Katz et al., 1981). The FST typically involves placing a mouse/rat in a cylinder of water (22–25°C). Then, following a defined period of struggle, the rodent becomes immobile or floats passively. In the case of the TST, a mouse is suspended by the tail about 35 cm above the floor. The mouse will quickly struggle to escape, but gradually stops and becomes immobile (Thierry et al., 1986). As a consequence of being placed in inescapable aversive conditions the rodents shift between periods of vigorous activity (e.g., searching behavior) and immobility (e.g., waiting behavior). The duration of immobility has been used as a measure of "behavioral despair" (Liu and Gershenfeld, 2003). While it may appear that such behaviors have little to do with models of depression, these models have been shown to have high (~90%) predictive success for structurally diverse compounds. In the chronic stress model, rats are exposed to a variety of stressors for 3 weeks, after which some rats are exposed to an acute stress followed by an open field test. Acutely stressed animals display high activity and low defecation in the open field compared to chronically stressed rats, unless the chronically stressed rats are given daily injections of an antidepressant (Pignatiello et al., 1989).

Forced Swim Test/Tail Suspension Test

Of relevance to hormetic dose responses is a report by Bai et al. (2001), who assessed the effects of imipramine on male C57Bl/6 mice using the FST. In this experiment the mice were placed in clear plastic cylinders (diameter 10 cm, height 25 cm) filled to 6 cm for 6 min. The duration of immobility was recorded during the final 4 min of the 6-min period of observation. The

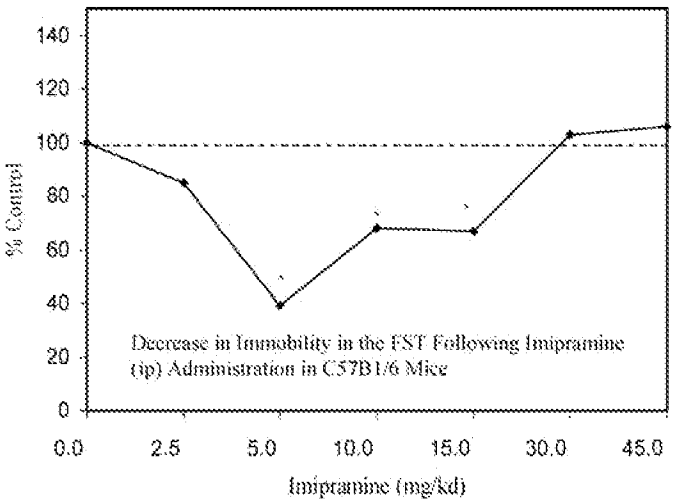

FIG. 69. Dose response curve of imipramine in the forced swim test (FST) in C57B1/6 mice. *Significantly different from controls at $p < .05$. (Source: Bai et al., 2001, Figure 2A, p. 189).

imipramine induced a U-shaped dose response (Figure 69). Tests with the NIH-Swiss mouse model yielded no indication of a U-shaped response with doses up to 45 mg/kg, suggesting that a broader dosing range might have been of value.

The research of Bai et al. (2001) is consistent with that of other reports indicating a U-shaped dose response with antidepressant drugs, initially demonstrated in animal model studies by Plotnikoff et al. (1971, 1973) and extended to depressed patients soon thereafter (Ehrensin and Kastin, 1974). Using an early version of the FST (see Porsolt et al., 1977, 1978; Nomura et al., 1982), Kastin et al. (1984) demonstrated that the peptide MIF-1 caused an increase in the number of rotations of the water wheel, representing a reversal of the immobility. The dose response followed the typical U-shaped dose-response relationship, with the maximum response being approximately twice that of the control value with the width of the stimulation dose range being about 10-fold. The clinical implications of the U-shaped dose response were addressed in a preliminary manner by Kastin et al., who suggested the need for caution when making determination of the effective dosage.

The FST and TST tests have also been used to assess the antidepressant activity of methylene blue (Eroglu and Caglayan, 1997) and extracts from aerial parts of hypericum perforatum (Butterweck et al., 1997, 1998a, 1998b). These investigations have also consistently revealed a U-shaped dose response using the FST over a broad dose range employing 5–7 doses per experiment (Figures 70–72). With respect to the research of Butterweck et al. (1997, 1998a, 1998b), the optimal dosage for antidepressant activity does not cause an increase in motor stimulation in the open field test and there was no change in activity of the rats during a subchronic treatment, thereby not supporting the conclusion that non-specific effects may cause the decreased immobility. Furthermore, use of D_2 and D_3 receptor

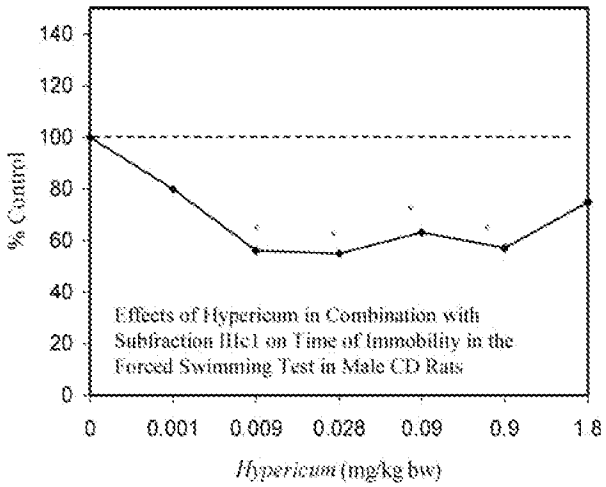

FIG. 70. An evaluation of hypericum on time of immobility in the forced swimming test in male CD rats. *Significantly different from controls at $p < .05$. (Source: Butterweck et al., 1998a, Figure 2, p. 292).

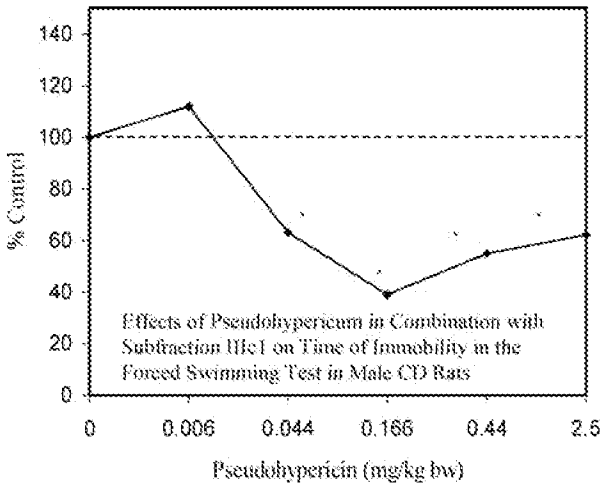

FIG. 72. An evaluation of pseudohypericin on time of immobility in the forced swimming test in male CD rats. *Significantly different from controls at $p < .05$. (Source: Butterweck et al., 1998a, Figure 2, p. 292a).

antagonists prevented the effect during the FST, suggesting a dopamine-mediated activity (Butterweck et al., 1997).

Learned Helplessness

Learned helplessness behavior using a shuttle-box task methodology has been shown to be regulated in a biphasic dose-response manner by corticosterone (Kademian et al., 2005). The dose response was U-shaped, showing the same quantitative features of the hormetic dose response. Possible underlying mechanisms to account for the U-shaped dose response were addressed in detail, although in a speculative fashion. However, in general, it appears that corticosterone can either increase or decrease the learned despair response via its effect on mineralocorticoid (MR) and glucocorticoid (GR) receptors in male Wistar rats that have had behavioral impairment induced by inescapable foot shocks. The foot shock caused the rat to become inactive, lacking any discernable movement (i.e., helplessness). Corticosterone concentrations that are able to affect a high percentage of MR depletion along with a moderate GR depletion are needed to avoid or lessen the learned helplessness. However, high levels of GR depletion are related to an increase of the helplessness behavior. The administration of the GR antagonist RU 38486 blocked the reduction of the helplessness behavior in adrenalectomized rats receiving 100 μg/ml corticosterone, emphasizing an important role for GR.

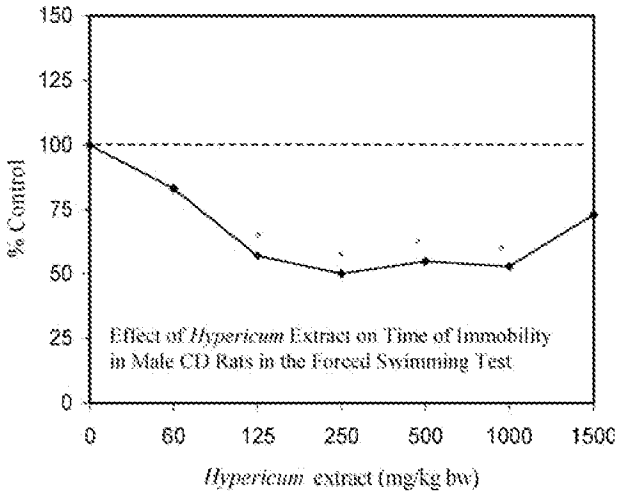

FIG. 71. The effect of hypericum extract in the forced swimming test in male CD rats. *Significantly different from controls at $p < .05$. (Source: Butterweck et al., 1997, Figure 8, p. 121).

Chronic Stress Test (CST)

The chronic stress test (CST) is a unique experimental paradigm to assess depression behavior. Over the course of 20 days, a series of stressors is given to the animals, with a different stressor given daily except for 2 days when no stressor is administered. The time of delivery of the stressor is also varied in order to maximize the unpredictable nature of the stresses. On day 21 (about 30 min into the dark cycle) the rats are placed into a lighted room for 1 h in the presence of a speaker emitting 95 dB white noise. After exposure to this noise/light stress, these rats are transported immediately to the open field test room. The open field testing is conducted in the dark (except for a 25-W light bulb) and with background noise (40-50 dB). Animals are placed facing one of four corners and observed for 3 min with four endpoints being scored (i.e., number of squares entered with four feet, latency to leave home square, defecation score, and latency to defecation). In this test Pignatiello et al. (1989) administered (ip) MIF-1 (prolyl-leucyl-glycinamide), an antidepressant, to male Sprague-Dawley rats on a daily basis

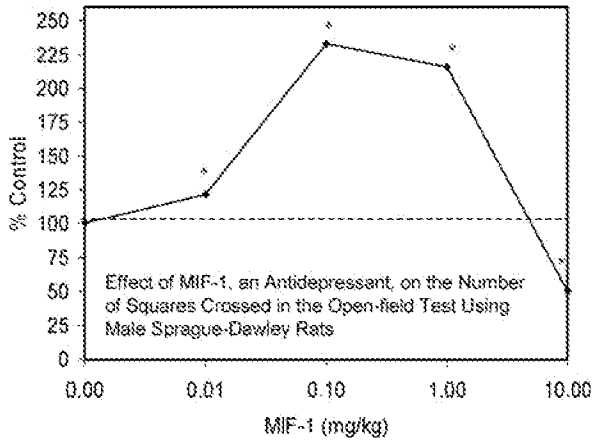

FIG. 73. Effect of MIF-1 on activity in an open-field maze as measured by the mean number of squares crossed. *Significantly different from controls at $p < .05$. (Source: Pignatiello et al., 1989, Figure 2, p. 739).

throughout the test period. The authors reported biphasic dose responses for squares crossed (Figure 73) and fecal boluses (Figure 74). Follow-up clinical research by this group reported an improvement in major depression following low subcutaneous doses of MIF-1 (Ehrensin, et al., 1994), consistent with the findings of the animal studies.

Anxiety/Ulcers (Communication Box Test and Immobilization/Cold Test)

In the mid 1980s the non-benzodiazepine buspirone generated considerable interest as a selective anxiolytic agent. Much of this interest was based on observations that buspirone did not cause cognitive impairment, physical sedation (Lader, 1982; Lucki et al., 1987), and positive reinforcement, as seen with

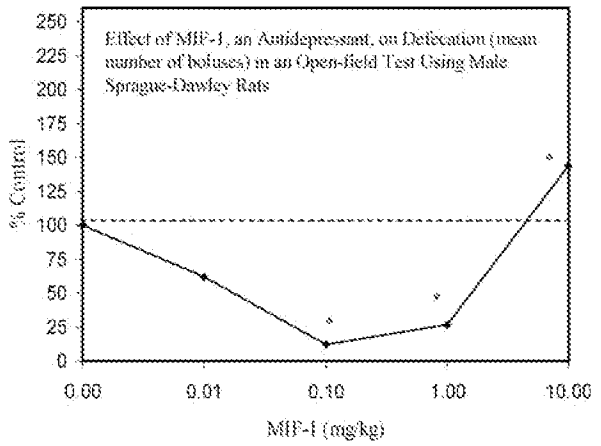

FIG. 74. Effect of MIF-1 on defecation (mean number of boluses) in an open-field maze. *Significantly different from controls at $p < .05$. (Source: Pignatiello et al., 1989, Figure 3, p. 740).

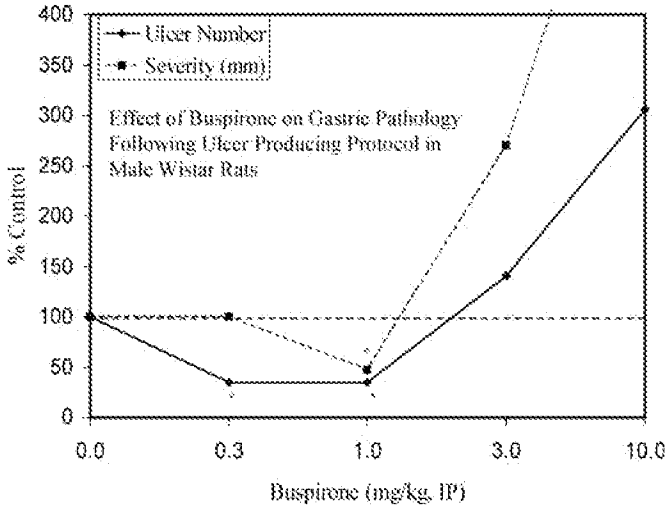

FIG. 75. Effect of buspirone on gastric pathology following ulcer producing protocol in male Wistar rats. *Significantly different from controls at $p < .05$. (Source: Sullivan et al., 1988, Table 1, p. 318).

other antianxiety drugs. Pharmacological assessment of buspirone suggested that its principal effects were probably mediated via dopaminergic pathways and that it may display properties of dopamine agonists and antagonists at different dose levels (Riblet et al., 1982; Skolnick et al., 1984).

Since buspirone displayed anxiolytic properties that were probably mediated by the dopaminergic system and since the administration of dopamine agonists and antagonists could decrease or increase stress-induced ulcers in rodents, Sullivan et al. (1988) assessed whether buspirone administration over a broad dose range in which it could act both as agonist (low doses) and antagonist (at high doses) could affect stress-induced ulcer formation in the adult male Wistar rat. Ulcers were induced quickly as a result of immobilization of the rats in Plexiglas restrainers at 4°C for 3 h. Control animals sacrificed immediately after the 3 h displayed over 6 (average of 6.2) gastric (stomach) ulcers per rat. The ulcers were also graded for severity based on size of the lesion. The buspirone, which was administered just prior to immobilization, was given over a 100-fold dose range using 5 doses (0.3–30.0 mg/kg) with semi-log dose spacing.

The buspirone induced a biphasic dose response in which low doses reversed both ulcer number and severity, while the reverse was the case at higher doses (Figure 75). The ulcer number and severity were reduced by about 50–60% with the low-dose treatment. These findings are consistent with observations that buspirone acts as both a DA agonist and antagonist depending on the dose. Further supporting this interpretation were data showing that haloperidol, a DA antagonist, and apomorphine, a DA agonist, respectively enhanced and reduced ulcer formation in the Sullivan et al. (1988) experimental setting.

Several years later it was reported that buspirone also displayed a biphasic dose response on ulcer formation in a mouse experimental anxiety model (Ogawa et al., 1993). This study induced ulcers via the so-called "communication box" method, which increased anxiety via an intraspecies emotion communication method. In this case two groups of mice are separated by transparent plastic board. The mice in the two groups could not contact each other but could see, hear, and smell each other. One group received footshocks of 10 s duration at an interval of 50 s, for 3 h, at 1.6 to 2.0 mA, which progressively increased by 0.2 mA per hour. This process was repeated with a new set of naive mice each day for 3 days. The group not receiving the footshocks was not changed but was able to receive the sensory cues given off by the shocked mice. Specialized control groups were concurrently run to control for experimental setting and feeding conditions. In a 3-day treatment protocol buspirone was administered to five different groups of mice with doses ranging from 1 to 25 mg/kg. As was the case in the Sullivan et al. (1988) study, a U-shaped dose response occurred with an optimal decrease in ulcer formation at 5 mg/kg. Of particular interest is that at 5 mg/kg the incidence of mice with ulcers decreased from 85% to 15%, a value that was even lower than in the sham control (30%).

While acknowledging the paper of Sullivan et al. (1988), Ogawa et al. (1993) hypothesized that the enhanced of toxicity at the higher dose may be due to either a hypothermic effect (Gudelsky et al., 1988) or its capacity to release corticosterone (Urban et al., 1986), as both of these effects can affect gastric ulcer formation (Brodie and Hanson, 1960; Brodie et al., 1961). No discussion was directed to the dopamine agonist/antagonist hypothesis of Sullivan et al. (1988).

GABA RECEPTOR COMPLEX (MECHANISTIC MODEL FOR MANY ANXIOLYTIC DRUGS)

GABA is the principal inhibitory neurotransmitter in the mammalian central nervous system. Its inhibitory actions are mediated by $GABA_B$ receptors that are associated with GTP-binding proteins and by $GABA_A$ receptors that have an integral anion channel. $GABA_A$ receptors are the site of action of numerous drugs of societal importance including benzodiazepines (BZ), barbiturates, steroids, convulsants, hypnotics, and ethanol. When GABA, or one of the drugs just noted, binds to its receptor, it enhances the flow of chloride ions through the channel and neuronal excitability is diminished in accordance with GABA's inhibitory neurotransmitter function. The cloning of more than a dozen $GABA_A$receptor subunits has provided a structural foundation for the pharmacological heterogeneity of GABA receptor systems with reference to the highly diverse central BZ-binding sites. Such structural developments provide a basis to assess interspecies and regional differences in the actions of various GABA ligands for a broad range of responses including those relating to hormetic-like dose-responses which result from allosteric modulation.

Benzodiazepines display their pharmacological effects in the brain by enhancing the actions of the inhibitory neurotransmitter GABA. The interaction of benzodiazepines with GABA involves the brain-specific drug receptors that are components of GABA receptors, including the GABA–benzodiazepine chloride ionophere receptor complex. The investigation of the drug receptor of GABA-regulated chloride ion channels was markedly enhanced in the early 1980s by Squires et al. (1983), who reported that labeled [^{35}S]-*t*-butylbicylophosphorothionate ([^{35}S]-TBPS), a derivative of a series of potent GABA antagonistic cage convulsants, binds with high affinity to brain-specific binding sites linked to GABA receptors. The use of TBPS in binding studies represented an important methodological advance over the use of the then only available ligand, dihydropicrotoxinin, which has high nonspecific binding to brain membrane fractions.

Using the cerebral cortex of the adult male Wistar rat, it was determined that the majority of GABA binding sites were constituents of the GABA–benzodiazepine receptor complex (Palacios et al., 1982; Supavilai and Karobath, 1984). Further investigations by Supavilai and Karobath (1984) determined that under specific membrane preparation conditions (e.g., reduced concentrations of NaBr or with its replacement by 100 *M* NaCl) TBPS binding displayed biphasic actions with muscimol, a GABA agonist, with high-affinity stimulation (EC50 23 n*M*) and low-affinity inhibition (IC50 724 n*M*). In the presence of a potent bicuculline-like GABA antagonist (i.e., R 5135), the biphasic response of muscimol on TBPS binding was consistently observed but shifted to the right with little impact on amplitude and width of the concentration stimulatory range. Similar findings of a biphasic dose response on TBPS binding were reported for diazepam (Figure 76), a response that was fully antagonized by the specific receptor antagonist Ro-15 1781 (Concas et al., 1990).

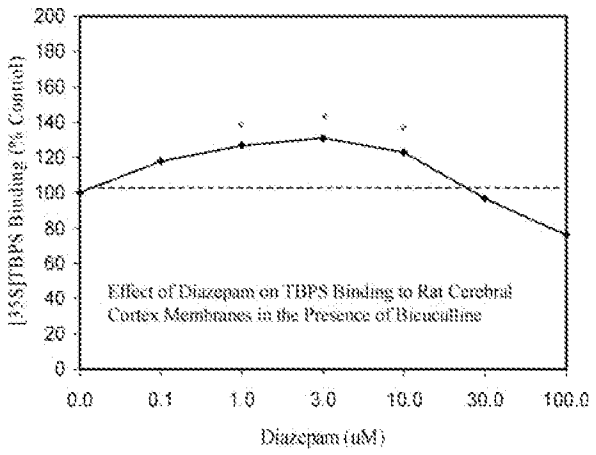

FIG. 76. Diazepam enhances [^{35}S]TBPS binding to unwashed membranes preparations in the presence of bicuculline. *Significantly different from controls at $p < .05$. (Source: Concas et al., 1990, Figure 1, p. 90).

Biphasic actions of etazolate and pentobarbital (e.g., both CNS depressants) on TBPS binding along with a Hill number >1 for their inhibitory effects led Supavilai and Karobath (1984) to suggest a multiplicity of TBPS binding sites in a GABA–benzodiazepine receptor complex with cooperativity between these sites. The findings of Supavilai and Karobath (1984) stimulated the development of research into allosteric mechanisms of GABA, typically using TBPS binding as the principal endpoint measured. The areas of research included the assessment of dose-response relationships for other GABA-receptor acting agents from different chemical classes, e.g., the synthetic steroids such as the anesthetic alphaxaline (Figure 77) (Turner et al., 1989) and some of its chemical relatives (e.g., various barbiturates such as the commonly studied pentobarbital; Turner et al., 1989; Liljequist and Tabakoff, 1986, 1993), numerous hypnotics (Lloyd et al., 1990), a broad range of glucocorticoids (Majewska, 1987), various insecticides (e.g., avermectin) (Figure 78) (Huang and Casida, 1997; Maksay and Van Rijn, 1993; Cully and Paress, 1991; Olsen and Snowman, 1982, 1983) and other agents, regional brain responses to such agents (Liljequist and Tabakoff, 1993; Srinivasan et al., 1999; Sapp et al., 1992), and the relationship of receptor subunit heterogeneity to account for both interspecies and regional GABA response differences (Sapp et al., 1992), drug interactions, and other modulatory influences such as the impact of prior seizures on subsequent dose-response relationships. Not only were allosteric effects reported with respect to TBPS binding but with other endpoints such as Cl-flux measures, which are highly correlated with the TBPS findings (Obata et al., 1988; Pomes, et al., 1994a, 1994b). In general, biphasic dose-response relationships were commonly reported with similar quantitative features in terms of the amplitude and range of the stimulatory response.

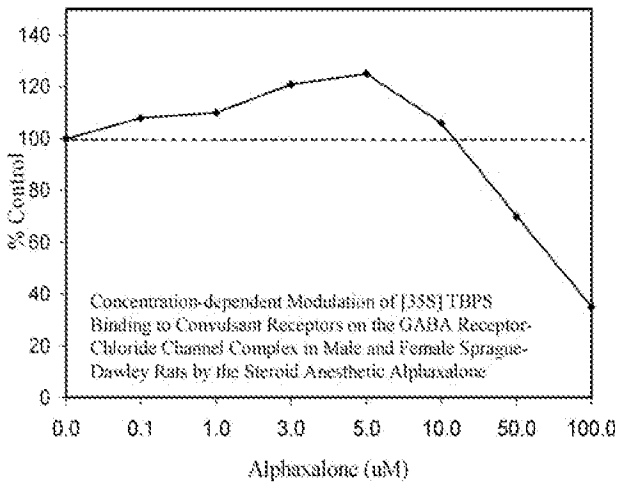

FIG. 77. Concentration-dependent modulation of [^{35}S]TBPS binding to convulsant receptors on the GABA receptor-chloride channel complex in male and female Sprague-Dawley rats by the steroid anesthetic alphaxalone. (Source: Turner et al., 1989, Figure 3, p. 962).

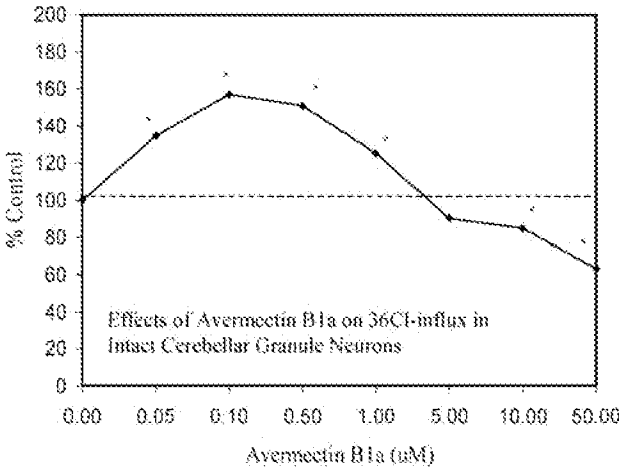

FIG. 78. Biphasic effects of AVM B_{1a} on ^{36}Cl-influx in intact cerebellar granule neurons. *Significantly different from controls at $p < .05$. (Source Huang and Casida, 1997, Figure 5, p. 264).

In terms of the different chemical classes that bind to the GABA receptor complex, it was typically found that each has a unique binding configuration with the receptor. Despite the specificity of binding properties within the GABA receptor complex, allosteric biphasic dose-response modulation was generally present. Follow-up research on the receptor complex via cloning technologies revealed that the functional $GABA_A$ receptor may be affected by multiple subunits, which determine receptor pharmacology, including specificity, modulatory responses such as allosteric behaviors and other features (Pregenzer et al., 1993).

Kinetic studies were also employed to assess the biphasic dose responses of the GABA agonists and antagonists. Maksay and Simonyi (1986) found that the biphasic dose responses (which they referred to a low-dose hooks) could be explained by an allosteric kinetic modulation of TBPS binding. The biphasic response occurs as a result of simultaneous but unequal acceleration of association and dissociation rate constants. In fact, model calculations in their paper utilizing modulation in the same direction of the on and off rates of binding can reproduce the observed biphasic phenomena.

Despite the consistency of the GABA-related biphasic dose-response phenomena and their allosteric modulatory mechanistic foundations, little discussion has focused on their biological/evolutionary significance. However, Majewska et al. (1986) opined that such biological changes are homeostatic and adaptive responses that permit endogenous regulatory factors to act in a coordinated fashion with positive and negative regulation of GABAergic neurotransmission. She further stated that the biological significance of a modest enhancement in the apparent affinity and density of convulsant-TBPS binding sites induced by physiologically meaningful concentrations of 17-OH-glucocorticoid is unknown, although it suggests that

glucocorticoids could play the role of a "second-order" modulator in which they may lower seizure threshold via the capacity to enhance the binding of model convulsants to TBPS binding sites.

GABA: Antidepressant

Exploration into the mechanism(s) by which antidepressants achieve their effect on mood is a central area of pharmacological research. When such investigations sought to determine the mechanism by which the antidepressant trizadone (TRZ) acts, an integrative hormetic mechanism was revealed. While TRZ has been an approved drug for nearly 50 years, its mechanism of action remained largely unresolved until the early 1990s when Marek et al. (1992) suggested that its antidepressant effects could be explained by its antagonism for the 5-$HT_{2/1C}$ receptor subtype. Subsequent findings added the complementary mechanism of a weak inhibition of the 5-HT-transporter (Frazer, 1997). Based on these mechanistic underpinnings, TRZ became classified as a SARI (i.e., serotonin-2 receptor transporter) (DeVane, 2000). These advances were able to explain how the blocking of the 5-HT_{2A} subtype receptor leads to a large increase in 5-HT extracellular levels, which is the necessary condition to cause the antidepressant effect. A key advance occurred when Cozzi and Nichols (1996) determined that GABAergic interneurons could be modulated by acting on the 5-$HT_{2A/2C}$ receptor subtype. These studies revealed that various 5-HT_{2A} antagonists inhibit K+-stimulated γ-GABA release from cortical slices, while the opposite effect occurred with 5-$HT_{2A/2C}$ agonist treatments (Abi-Saab et al., 1999). These convergent developments led to the possibility that TRZ may achieve the large increase in 5-HT extracellular levels (and its antidepressant effect) via the modulation of GABA extracellular levels when the 5-HT_{2A} receptor is blocked.

Using cortical slices of male Sprague-Dawley rats, Luparini et al. (2004) reported that TRZ administration caused a biphasic dose response of GABA release, with low concentrations (10^{-10}–$10^{-7}M$) being inhibitory while higher concentrations (10^{-6}–$10^{-4}M$) enhanced GABA release. 5-HT release was increased across the entire range of TRZ concentrations tested. However, the increase was rapid (12 to 16 min) after high concentrations but delayed (observed after 24 to 28 min) after low concentrations. Follow-up microdialysis studies revealed the biphasic dose response in which TRZ (sc) decreased GABA extracellular concentrations at low doses while increases occurred at higher doses (Figure 79). Administration of 5-HT_{2A} antagonists reduced GABA release while effecting a delayed increase in 5-HT extracellular levels.

These findings led to the recognition that TRZ affects a reciprocal modulation between the corticol serotonergic and GABAergic systems. At low concentrations TRZ treatment reduces GABA release by blocking 5-HT_{2A} receptors on GABA neurons. This reduction in GABA release is associated with an increase in 5-HT release. However, when TRZ concentrations are high, this treatment increases 5-HT release by inhibiting the

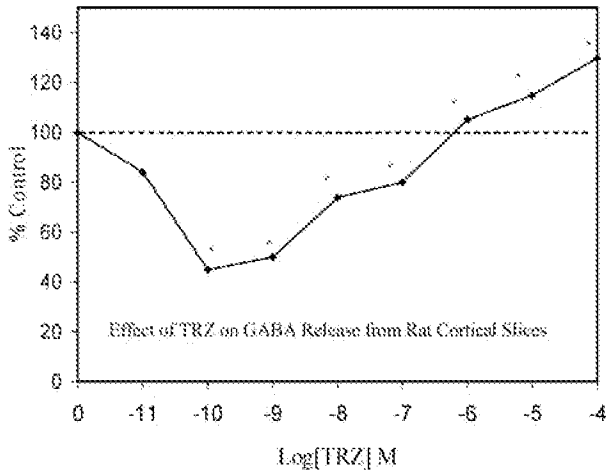

FIG. 79. Effect of TRZ on GABA release from rat cortical slices. Peak percent changes in GABA release in the concentration range of 10^{-11}–$10^{-4}M$. *Significantly different from controls at $p < .05$. (Source: Luparini et al., 2004, Figure 3B, p. 1121).

5-HT transporter. While the increase in 5-HT release is a necessary condition for the antidepressant effect of TRZ, the most likely mechanism appears to be via 5-HT_{1A} receptor downregulation (Subhash et al., 2002).

The quantitative features of the biphasic dose response are consistent with the hormetic response. TRZ affects a decrease in GABA release over approximately a 1,000- to 10,000-fold range. The magnitude of the decrease was about 35–40% at the maximum. However, at high doses the responses were reversed (Figure 79).

The relationship between TRZ and the hormesis concept is both interesting and complex. The relationship to hormesis is seen with it effects on GABA release which is clearly biphasic. GABA enhances the release of 5-HT by different mechanisms at low and at high concentrations. Thus, the antidepressant effect of TRZ would be effective over a very broad dose range. There appears to be no significant difference in the percent increase of 5-HT release via these two mechanisms, suggesting similar drug-induced responses at low and high TRZ doses.

Biphasic Dose Response—Partial Agonist Strategy

The recognition that drugs could biphasically affect TBPS binding to cortical tissue has led to several research directions. Of particular relevance have been pharmaceutical initiatives to explore this observation in the discovery of a new class of BZR ligands that effectively reduce anxiety but with fewer adverse side effects.

Ligands that bind to the benzodiazapine site display a continuum of intrinsic activity, with some being full agonists (displaying anxiolytic, hypnotic, and anticonvulsant activity), antagonists (i.e., no activity), and inverse agonists (i.e., displaying proconvulsant and anxiogenic activities). As implied from these

effects, antagonists have little or no effect on the chloride flux whereas inverse agonists decrease the flow of the chloride ion. Given this continuum of activity, partial agonists act within this efficacy spectrum, leading to the hypothesis that partial agonists may display antianxiety properties.

It was hypothesized by Jacobsen et al. (1999) that with proper adjustment of intrinsic activity, a therapeutic agent with biphasic efficacy could be synthesized, having characteristics that limits its own agonistic effects over a broad dose range, thereby reducing its abuse potential. This general perspective fueled the search for viable partial agonists (Im et al., 1995; Jacobsen et al., 1996a, 1996b, 1999; Mickelson et al., 1996).

In addition to the discovery and characterization of partial BZR agonists, GABA-A receptor channel complexes were determined to exist as composites of different receptor subunits ($\alpha, \beta, \gamma, \delta$). The identification of subtype selective ligands also permits novel opportunities to discriminate between useful anxiolytic or hypnotic effects and harmful side effects.

In the search for BZR partial agonists, Jacobsen et al. (1999) explored a series of piperazine ureas, many of which were found to be partial agonists based on the TBPS binding and/or Cl-current ratio assays. Such investigations also yielded a subclass of compounds that showed biphasic effects in in vitro assays, along with progressively greater antagonistic activity at higher drug concentrations.

The investigations of Jacobsen et al. (1996a, 1996b, 1999) revealed that many quinoxaline piperazine ureas displayed biphasic dose responses in in vitro bioassays, one of which they highlighted in an earlier paper by Im et al. (1995). The model agent (i.e., U-P7775 in Im et al., 1995; or compound 47 in Jacobsen et al., 1999) is a GABA-A receptor ligand exhibiting dual functionality based on the capacity to affect GABA-induced Cl^- currents with human embryonic kidney cell lines (HEK- 293) expressing various GABA receptor subtypes. The agents induced a biphasic dose-response profile in the $\alpha 1\beta 2\gamma 2$ receptor phenotype, with low concentrations enhancing the Cl-current with a maximum of about 220%. The stimulatory, but not the inhibitory, response of U-97775 was blocked by the benzodiazapine antagonist Ro-15-1788, suggesting that this ligand interacts at a second site on GABA receptor, which is separate from the benzodiazepine site. Similar biphasic dose responses and antagonist blocking were also reported for TBPS binding. Testing of other receptor subtypes (i.e., $\alpha 3\beta 2\gamma 2$) also yielded similar biphasic dose responses.

In their assessment, Im et al. (1995) noted that drugs with dual functionality, such as U-97775, are likely to be commonly observed, considering the presence of multiple binding sites within the same receptor complex as is the case with GABA-A. Not only is this the case with the synthetic benzodiazapine ligands, but also for the 5HT3 antagonist ICS 205-930, which induced biphasic dose responses with the GABA-invoked Cl^- currents that required the $\gamma 2$ receptor subunit, a response that was again blocked by the benzodiazapine antagonist Ro-15-1788 (Klien et al., 1994).

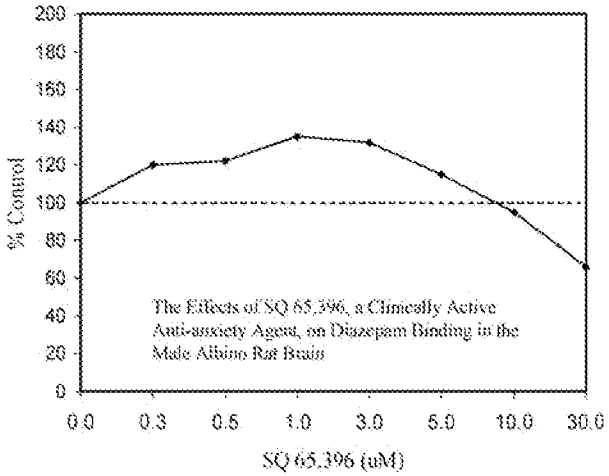

FIG. 80. Effects of SQ 65,396 on ^{3}H-diazepam binding in rat brain. (Beer et al., 1978, Figure 1, p. 850).

A similar dose response has been reported with Sq 65,396 (a pyrazolo (3,4,6) pyridine), an anti-anxiety agent that acts by biphasically affecting the binding of diazepam in the mouse cortex (Beer et al., 1978) (Figure 80). The mechanism by which this agent enhanced the binding of diazepam was via an increase in binding affinity without affecting the number of receptors.

The concept espoused by Jacobsen et al. (1996a, 1996b, 1999) of optimized partial agonists that have a broader therapeutic zone with fewer side effects applies not only to synthetic drugs, as in the case of anxiolytic drugs, but also for endogenous agonists as a common adaptive strategy. For example, biphasic dose responses have been reported for the neurosteroid 3αOH-DHP (allopregnanolone) on the recombinant $\alpha 6\beta 2\gamma 2$ GABA-A receptor complex in the brain (Majewski and Schwartz, 1987; Zaman et al., 1992; Hauser et al., 1995). 3α-OH-DHP and pregnanolone sulfate (PS) in the lower nanomolar range enhanced GABA-induced currents but were antagonists in the upper nanomolar and micromolar range. Similar biphasic responses were also reported for the neuro-steroid, tetrahydrodeoxycorticosterone (THDOC) in assessments of GABA-activated Cl^- currents in cultured rat hypothalamic neurons (Wetzel et al., 1999).

Environmental Applications

The organochlorine pesticides belonging to the polychlorocycloalkanes (PCCAs) are well known for their capacity to induce grand mal convulsions in mammals as a principal neurotoxin effect. Agents of this class that may induce such convulsions include lindane, aldrin, and dieldrin. In contrast, some hexachlorocyclohexane isomers such as β- and-γ-HCH are known as CNS depressants.

Since PCCAs are known to interact with the $GABA_A$ receptor, Pomes et al. (1994a, 1994b, 1994c) explored the effect of convulsant and nonconvulsant organochlorine compounds on GABA-induced ^{36}Cl flux in a homogeneous cortical neuronal population from 15-day-old fetal mice (of one mouse strain) that

expresses the $GABA_A$ receptor. The findings revealed that the convulsant-inducing agents (i.e., lindane, endosulfon, and dieldrin and aldrin) displayed a concentration-dependent decrease in the ^{36}Cl uptake. In contrast, the depressant activity agents (i.e., β- and γ-HCH) induced a biphasic concentration response, with stimulation occurring at the lowest two concentrations, while an inhibitory response occurred at the three highest concentrations. The maximum stimulatory responses were 40–45% for both agents.

The authors hypothesized that the increase of GABA-induced Cl-flux by β- and γ-HCH may account for the depressant activity of those agents and that the biphasic response could be accounted for as a balance between indirect (via an increase of intracellular Ca^{2+}) and direct effects of γ-HCH on GABA-induced ^{36}Cl uptake. They noted that other depressant drugs such as BZs and pentobarbital display biphasic effects on the $GABA_A$ receptor (Allan and Harris, 1986; Obata et al., 1988).

The findings of Pomes et al. (1994a, 1994b, 1994c) represent the first reports of both different and opposite effects between the convulsant (α-HCH-lindane) and the depressant (γ-HCH) isomers on GABA-induced Cl uptake, mostly likely due to the use of considerably higher and non-physiological concentrations (see Fishman and Gianutsos, 1988).

These findings led to a wide range of possible explanations, all of which were highly speculative, for example, that the low-dose stimulation may not be due to how these agents cause depressant effects but how they avoid convulsant effects. The lack of a biphasic response in the convulsant drug may be because the dose was too high. In the study of biphasic dose responses in the GABA system, authors have typically used TBPS binding as the principal endpoint. However, the Cl flux also commonly yields a biphasic dose response. It should be noted that both of theses parameters were shown to be highly correlated with each other in the same system (Pomes et al., 1994a, 1994b, 1994c).

DISCUSSION

The present assessment has evaluated the principal anxiolytic drug screening protocols and testing procedures in order to determine the nature of the dose response for agents active in these tests. These screening tests included the elevated plus maze test, open field test, social interaction test, hole-board test, staircase test, light–dark test, four plates test, Vogel's conflict test, and various tests of depression such as the forced swim test, the tail suspension test, and the freeze behavior test and several ulcer-inducing tests such as the communication box test and the immobilization/cold stress test. Tests were selected for evaluation based on considerations that included experimental validation, susceptibility to false positives and negatives, widespread acceptance/use, possible intertest comparisons, study design, statistical power strengths, mechanism-oriented research, and inclusion of protocols that were robust with respect to number of doses used and their spacing. Each test was evaluated within its historical developmental context as well as within current frameworks that reflect iterative modifications of such tests since their initial formulation.

Hormetic Dose-Response Features

This assessment has revealed that anxiolytic drugs commonly display hormetic-like biphasic dose responses, independent of the test and animal model employed. The quantitative features of the dose responses across the various anxiolytic testing schemes are remarkably similar with respect to the maximum increase and width of the stimulatory response. Consistent with hormetic response features, the maximum responses are generally modest with most being less than twice that of the control, with the average maximum value being about 30–60% greater than control values. The width of the stimulatory response, while variable at times, is typically over a range from about 1/5 to 1/30 of the zero equivalent point or the traditional threshold response. While numerous investigators in the field of anxiety-related research have been aware of the biphasic nature of the dose response, there seems to have been little explicit appreciation of its widespread occurrence and similar features of the dose response across tests, models, and chemical classes.

Ceiling Effect and Drug Potency

The quantitative aspects of the dose response suggest that maximum anxiolytic responses may be subject to and/or expressions of the so-called "ceiling effect," which is common and a broadly generalizable characteristic of pharmacological responses. There is therefore a remarkable quantitative consistency with respect to the maximum anxiolytic response. This is the case regardless of the potency of the agent. That is, highly potent drugs may show anxiolytic effects at very low doses relative to less potent agents, but the quantitative features of the dose responses between very potent and less potent agents are strikingly similar. This observation has been consistently seen across the broad spectrum of hormetic dose responses regardless of biological model, endpoint measured, and chemical class. Thus, it is likely that the ceiling effect is a component of the hormetic dose response.

High Dose Inhibition Explanation

Explanations for the decrease in the response at the higher doses can be complex and may vary according to agent and/or model. While it has been at times assumed that decreased responses at higher doses may result from possible drug sedative properties, it has been established that various drugs display their high-dose decreases in the absence of sedative effects. In such studies it has often been the case that the decrease in response may be blocked by a receptor agonist or antagonist, thereby decoupling the decrease from drug-enhanced sedative effects and linking the decrease to specific receptor-based mediated effects (Pick et al., 1996, 1997; Milman et al., 2006).

Inter-Anxiolytic Test Comparisons

A number of publications have compared the response of the same drug in multiple screening tests (e.g. EPM, L-D test, open

TABLE 2
Receptor pathway for hormetic responses

Screening test	Agonist	Receptor	Antagonists/ blocking agent	Reference
Pain	PGE_2	NMDA	Multiple antagonists	Nishihara et al., 1995
Forced swimming	Hypericum extracts	Dopamine	Multiple D_2 and D_3 antagonists	Butterweck et al., 1997
Vogel's conflict test	Amperozide	BDZ	Bicuculline	Engel et al., 1989
Attention deficit disorder	Methylphemidate	α2-adrenoreceptor	Idazoxan	Arnsten and Dudley, 2005
EPM	CPA	Adenosine	CPX blocked effect of CPA	Jain et al., 1995
SIT	Chlordizaepoxide, Nitrous oxide	BDZ	Flumazenil	Quock et al., 1993
Staircase test	Alprazolan	BDZ	Flumazenil	Pick et al., 1996, 1997
Epileptic model/seizure	Morphine	Opiate	Naloxone	Lauretti et al., 1994
Light–dark test	Buspirone, Sulpiride	Dopamine	Apomorphine	Pich and Samanin, 1986
EPM	dl-THP	BDZ	Multiple antagonists	Leung et al., 2003
Pain	Apomorphine	Dopamine, D_2	Antagonist s-(–)-sulpiride	Pelissier et al., 2006
Learned helplessness	Corticosterone	Glucocirtuciud receptors	GR depletion (RU 38486)	Kademian et al., 2005
Freezing effect	Spermidine	NMDA	Antagonist arcame	Rubin et al., 2004
Self grooming	m-CPP	$5\text{-}HT_{2C}$	Multiple antagonists	Graf et al., 2003

field test, and others). While these findings can often present some measure of complexity, there has been a general observation that a drug that displays an anxiolytic effect in one screening test may very likely do so in other screening tests, assuming that the same experimental procedures are followed. While there have been numerous examples of quantitative differences in hormetic responses with the same drug in different tests, a careful examination of the respective dose-response relationships reveals various agents may display subtle but important differences in dose-response features. For example, in a comparison of *dl*-THP in the EPM and hole-board tests, Leung et al. (2003) reported that this agent induced stimulatory responses at a lower dose in the EPM than in the hole-board test, while the width of the stimulation in absolute terms was similar between the two tests (Figure 25, hole-board test section). This would suggest that the two tests may be measuring closely related but not identical processes.

Common Downstream Mechanism

Numerous investigators have provided information on the mechanism of action of anxiolytic agents based upon the application of receptor blocking techniques, typically via the use of synthetic agonists and antagonists. Table 2 lists numerous receptor-based pathways by which hormetic-like biphasic dose responses were reported for anxiolytic endpoints. These receptors include the benzodiazepines, adenosine, dopamine, opiates, NMDA, 5HT, glucocorticoids, and the alpha-2 adrenoreceptor. By blocking the pathway activated by these receptors, anxiolytic effects induced by many different agents have been prevented and then linked mechanistically back to a specific receptor pathway. Other receptor-mediated pathways have been identified as essential for agonist-induced anxiolytic effects but did not present experiments with receptor blocking agents. In this respect it is interesting to note that approximately 12 different receptor pathways have been shown to facilitate the occurrence of a low-dose enhancement of the anxiolytic effect, with each activated pathway leading to an anxiolytic response with similar quantitative features of the dose response. This suggests that factors determining the quantitative features of the dose response may occur at a downstream convergence site. This convergence site concept suggests the existence of a type of metabolic carousel in which the receptor pathway leads to a type of "central station" integration process center.

Why Endogenous Agonists Have Few Side Effects

There has been some discussion and analysis to develop a biologically based framework to account for the quantitative features of the dose response. As discussed in the GABA agonist section, there may be considerable advantages for endogenous ligands to be partial agonists with dual functionality that could

limit one's agonist activity and affect a biphasic dose-response relationship. This was seen as a possible way to limit undesirable drug side effects while at the same time broadening the concentration range over which an optimized response could occur (Im et al., 1995; Jacobsen et al., 1996a, 1996b, 1999). While these authors have utilized this concept in a search for partial GABA agonists that might display biphasic dose responses, their conceptual framework could be generalized to a broad spectrum of endogenous ligands and maybe a strategy for minimizing the occurrence of side effects under routine, not exogenous exposure, situations.

Anxiolytic Drugs, Self-Reenforcement, and Hormesis

While this article has principally assessed the dose responses of anxiolytic drugs within the context of animal model screening tests, it is important to recognize that anxiolytic agents can be drugs of abuse. Consequently, they have been extensively assessed with respect to intravenous self-administration and schedules of reinforcement within the context of addictive behavior. As early as 1955, Dews demonstrated the importance of ongoing behavior in determining drug effects. In his studies, pigeons were trained to peck a key under two different intermittent schedules of food presentation: a fixed interval (i.e., food offered after a specific duration) or fixed ratio (i.e., food offered after a certain number of pecks, independent of time). After the responding by the pigeons had become stable on both schedules (fixed interval/ratio), a broad range of pentobarbital doses was administered. In this study, fixed-ratio responses were depressed by 4.0 or 5.6 mg pentobarbital/bird. While fixed-interval responding was also markedly diminished, the reduction started at lower doses (1.0 mg/bird). Furthermore, the fixed-ratio responding was increased by doses of pentobarbital that decreased fixed interval responding. Further lowering of the doses (i.e., down to 0.25–0.5 mg/bird) induced increased rates of responding on both schedules.

The experiments of Dews revealed that the effects of a drug can depend on the type of ongoing behavior. This behavior is likewise contingent on the schedule of the reinforcement that sustains that behavior. A similarly important observation by Dews was that the drug's effect depends on drug dose. These findings had a major impact on the field of psychopharmacology, leading to widespread recognition that many drugs display an inverted U-shaped dose response relationship with a stimulatory effect at low doses and inhibitory effects at higher doses, but nevertheless occurring within a dose range that can alter behavior while not inducing anesthesia, ataxia, or general toxicity (Seiden and Dyskra, 1977).

While classes of drugs (e.g., psychomotor stimulants, antipsychotics, narcotic analgesics, narcotic antagonists, tricyclic antidepressants, hallucinogens, cholinergic blockers, cholinergic agonists) need to be separately evaluated, antianxiety agents (e.g., amobarbital, pentobarbital, phenobarbital, chlordiazepoxide, diazepam, and oxazepam) have been generally recognized as displaying an inverted U-shaped dose response, independent of the type of schedule of reinforcement: i.e., fixed interval (Dews, 1955, 1964; Kelleher et al., 1961; Richelle et al, 1962; Cook and Kelleher, 1962; Laties and Weiss, 1966; Bignami and Gatti, 1969; Wuttke and Kelleher, 1970; McMillan, 1973), fixed ratio (Dews, 1955; Kelleher et al., 1961; Morse, 1962; Cook and Catania, 1964; Waller and Morse, 1963; Rutledge and Kelleher, 1965; Wedeking, 1968, 1974), differential reinforced low rate (DRL) (i.e., like an FI schedule in that only the first response after a specific interval is reinforced; however, no response may occur during the interval; responding on the DRL is typically measured in terms of inter-response times) (Kelleher et al., 1961; Morse, 1962; Richelle et al., 1962; McMillan and Campbell, 1970; Sanger et al, 1974; Sanger and Blackman, 1975), and variable interval (Kelleher et al., 1961; Morse, 1962; Hanson et al, 1967; Wedeking, 1974) (Seiden and Dyskra, 1977). The quantitative features of these dose responses are generally similar with respect to the width and maximum stimulatory response and relationship to the zero equivalent point. These quantitative dose-response features are also common among drugs of other classes, e.g., amphetamine (Byrd, 1973; Gonzalez and Goldberg, 1977; McMillan, 1969; clozapine (Vanover et al., 1993), cocaine (Howell and Byrd, 1991, 1995; Kuzmin et al., 1992), morphine (Kuzmin et al., 1996; Spealman et al, 1977, 1979, 1989), and nicotine (Risner and Goldberg, 1983), that typically display inverted U-shaped dose responses during assessment of patterns of reinforcement behavior. The adoption of a similar quantitative dose-response strategy independent of biological model, endpoint, chemical class, and even schedule of reinforcement behavior represents a very generalized pattern of responsiveness that has been virtually unrecognized and not addressed in the biomedical, physiological, and evolutionary oriented sciences.

Anxiolytic and Anxiogenic Effects

In the assessment of screening tests for anxiolytic drugs, it is generally observed that anxiety is reduced at low while being increased at higher doses. The same drug therefore is capable of causing both a reduction and an increase in anxiety depending on the dose. On occasion there are situations in which at low doses there are increases in anxiety while at higher doses anxiolytic effects occur. The quantitative features of the dose response in the low dose zone are similar to those seen for the anxiolytic response. Likewise, there are circumstances (e.g., Substance P) in which an agent induces the anxiolytic response at low doses whereas in another species the process is reversed such that an anxiogenic response becomes the low-dose effect. In these cases, the dose-response features are fully consistent with the hormetic dose-response model.

SUMMARY

Despite the fact that hormetic-like biphasic dose-response relationships are quite common and expected in the assessment of anxiety responses, the present article represents the first attempt to integratively document and assess such observations. In fact, the lack of such dose-response assessment and integration is not unique to this area of experimental psychology

but has also been shown to occur in areas of the biomedical sciences such as immunology (Calabrese, 2005a), cancer cell biology (Calabrese, 2005b), toxicology (Calabrese, 2005c; Calabrese and Baldwin, 2001, 2003), and others. Researchers in these areas have certainly recognized biphasic dose responses in their findings, and searched for mechanistic understandings and applications in clinical domains (Calabrese, 2008, Calabrese et al., 2007). However, there has been a general lack of appreciation that highly diverse biological systems display dose-response relationships that are quantitatively similar and with similar underlying general mechanistic strategies that achieve the hormetic dose response.

REFERENCES

Abi-Saab, W.M., Bubser, M., Roth, R.H., and Deutch, A.Y. (1999). 5-HT_2 receptor regulation of extracellular GABA levels in the prefrontal cortex. *Neuropsychopharmacology* **20**:92–96.

Allan, A.M., and Harris, R.A. (1986). Anesthetic and convulsant barbiturates alter γ-aminobutyric acid-stimulated chloride flux across brain membranes. *J. Pharmacol. Exp. Ther.* **238**:763–768.

Araujo, J.E., Huston, J.P., and Barndao, M.L. (1998). Aversive effects of the C-fragment of substance P in the dorsal periaqueductal gray matter. *Exp. Brain Res.* **123**:84–89.

Arnsten, A.F.T., and Dudley, A.G. (2005). Methylphenidate improves prefrontal cortical cognitive function through α2 adrenoceptor and dopamine D1 receptor actions: Relevance to therapeutic effects in attention deficit hyperactivity disorder. *Behav. Brain Funct.* **22**;1(1):2.

Aron, C., Simon, P., Larousse, C., and Boissier, J.R. (1971). Evaluation of a rapid technique for detecting minor tranquilizers. *Neuropharmacology* **10**:459–469.

Artaiz, I., Romero, G., Zazpe, A., Monge, A., Caldero, J.M., Roca, J., Lasheras, B., and Del Rio, J. (1995). The pharmacology of VA21B7: an atypical 5-HT_3 receptor antagonist with anxiolytic-like properties in animal models. *Psychopharmacology* **117**:137–148.

Bai, F., Li, X., Clay, M., Lindstrm, T., and Skolnick, P. (2001). Intra- and interstrain differences in models of "behavioral despair." *Pharm. Biochem. Behav.* **70**:187–192.

Baldwin, H.A., Johnston, A.L., and File, S.E. (1989). Antagonistic effects of caffeine and yohimbine in animal tests of anxiety. *Eur. J. Pharmacol.* **159**:211–215.

Baumann, S.W., Baur, R., and Sigel, E. (2003). Individual properties of the two functional agonist sites in GABA(A) receptors. *J. Neurosci.* **23**:11158–11166.

Baur, R., and Sigel, E. (2003). On high- and low-affinity agonist sites in GABA-A receptors. *J. Neurochem.* **87**:325–332.

Beck, C.H., and Fibiger, H.C. (1995). Chronic desipramine alters stress-induced behaviors and regional expression of the immediate early gene, c-fos. *Pharmacol. Biochem. Behav.* **51**:331–338.

Beer, B., Klepner, C.A., Lippa, A.S., and Squires, R.F. (1978). Enhancement of ^{3}H-diazepam binding by SQ 65,396: A novel anti-anxiety agent. *Pharmacol. Biochem. Behav.* **9**:849–851.

Belzung, C., Misslin, R., Vogel, E., Dodd, R.H., and Chapouthier, G. (1987). Anxiogenic effects of methyl-B-carboline-3-carboxylate in a light/dark choice situation. *Pharmacol. Biochem. Behav.* **28**:29–33.

Bertaina-Anglade, V., La Rochelle, C.D., and Scheller, D.K.A. (2006). Antidepressant properties of rotigotine in experimental models of depression. *Eur. J. Pharmacol.* **548**:106–114.

Bignami, G., and Gatti, G.L. (1969). Analysis of drug effects on multiple fixed ratio 33-fixed interval 5-min in pigeons. *Psychopharmacologia* **15**:310–332.

Boissier, J.-R., and Simon, P. (1962). La reaction d'exploration chez la souris. *Therapie* **17**:1225–1232.

Boissier, J.-R., and Simon, P. (1964). Dissociation de deux compesantes dans le compartement d'investigation de la souris. *Arch. Int. Pharmacodyn.* **147**:372–387.

Boissier, J.R., Simon, P., and Aron, C. (1968). A new method for rapid screening of minor tranquillizers in mice. *Eur. J. Pharmacol.* **4**:145–151.

Boissier, J.R., Simon, P., and Soubrie, P. (1976). New approaches to the study of anxiety and anxiolytic drugs in animal. *Int. Congress of Pharmacology,* pp. 213–222. Pergamon Press.

Borsini, F., Podhorna, J., and Marazziti, D. (2002). Do animal models of anxiety predict anxiolytic-like effects of antidepressants? *Psychopharmacology* **163**:121–141.

Bourin, M. (1997). Animal models of anxiety: Are they suitable for predicting drug action in humans? *Pol. J. Pharmacol.* **49**:79–84.

Bourin, M., and Hascoet, M. (2003). The mouse light/dark box test. *Eur. J. Pharmacol.* **463**:55–65.

Bourin, M., Chenu, F., Ripoll, N., and David, D.J.P. (2005). A proposal of decision tree to screen putative antidepressants using forced swim and tail suspension tests. *Behav. Brain Res.* **164**:266–269.

Britton, D.R., and Britton, K.T. (1981). A sensitive open field measure of anxiolytic drug activity. *Pharmacol. Biochem. Behav.* **15**:577–582.

Brodie, D.A., and Hanson, H.M. (1960). A study of the factors involved in the production of gastric ulcers by the restraint technique. *Gastroenterology* **38**:353–359.

Brodie, D.A., Marshall, R.W., and Moreno, O.M. (1961). The effect of restraint stress and gastric acid secretion in the chronic gastric fistula rat. *Physiologist* **4**:14.

Burgess, H.J., Fogg, L.F., Young, M.A., and Eastman, C.I. (2004). Bright light therapy for winter depression—Is phase advancing beneficial? *Chronobiol. Int.* **21**:759–775.

Butterweck, V., Petereit, F., Winterhoff, H., and Nahrstedt, A. (1998a). Solubilized hypericin and pseudohypericin from *Hypericum perforatum* exert antidepressant activity in the forced swimming test. *Planta Med.* **64**:291–294.

Butterweck,V., Wall, A.U., Lieflander-Wulf, U., Winterhoff, H., and Nahrstedt, A. (1998b). Effects of the total extract and fractions of *Hypericum perforatum* in animal assays for antidepressant activity. *Psychopharmakotherapie* **5**:56–62.

Butterweck, V., Wall, A., Lieflander-Wulf, U., Winterhoff, H., and Nahrstedt, A. (1997). Effects of the total extract and fractions of *hypericum perforatum* in animal assays for antidepressant activity. *Pharmacopsychiatry* **30**(Suppl.):117–124.

Byrd, L.D. (1973). Effects of d-amphetamine on schedule-controlled key pressing and drinking in the chimpanzee. *J. Pharmacol. Exp. Ther.* **185**:633–641.

Calabrese, E.J. (2005a). Hormetic dose-response relationships in immunology: Occurrence, quantitative features of the dose-response, mechanistic foundations and clinical implications. *Crit. Rev. Toxicol.* **35**:89–306.

Calabrese, E.J. (2005b). Cancer biology and hormesis: human tumor cell lines commonly display hormetic (biphasic) dose responses. *Crit. Rev. Toxicol.* **35**:463–582.

Calabrese, E.J. (2005c). Paradigm lost, paradigm found: The re-emergence of hormesis as a fundamental dose response model in the toxicological sciences. *Environ. Pollut.* **138**:378–411.

Calabrese, E.J. (2008). Hormesis: Why it is important to toxicology and toxicologists. *Env. Toxicol. Chem.* **27**:1451–1474

Calabrese, E.J., and Baldwin, L.A. (2003). The hormeitc dose response model is more common than the threshold model in toxicology. *Tox. Sci.* **71**:246–250.

Calabrese, E.J., and Baldwin, L.A. (2001). The frequency of U-shaped dose-responses in the toxicological literature. *Tox. Sci.* **62**:330–338.

Calabrese, E.J., and Baldwin, L. (1997). The dose determines the stimulation (and poison): Development of a chemical hormesis data base. *Int. J. Toxicol.* **16**:545–559.

Calabrese, E.J., and Blain, R. (2005). The occurrence of hormetic dose responses in the toxicological literature, the hormesis database: An overview. *Toxicol. Appl. Pharmacol.* **202**:289–301.

Calabrese, E.J., Bachmann, K.A., Baile, A.J., Bolger, P.M., Borak, J., Cai, L., Cedergreen, N., Cherian, M.G., Chiueh, C.C., Clarkson, T.W., Cook, R.R., et al. (2007). Biological stress response terminology: Integrating the concepts of adaptive response and preconditioning stress within a hormetic dose-response framework. *Toxicol. Appl. Pharmacol.* **222**:122–128.

Cao, B-J., and Rodgers, R.J. (1997). Influence of 5-HT_{1A} receptor antagonism on plus-maze behaviour in mice. II. WAY 100635, SDZ 216-525 and NAN-190. *Pharmacol. Biochem. Behav.* **58**:593–603.

Cechin, E.M., Quevedo, J., Barichello, T., Machado-Vieira, R., Gentil, V., and Kapczinski, F. (2003). Dose-related effects of propericiazine in rats. *Braz. J. Med. Biol. Res.* **36**:227–231.

Charney, D.S., Heninger, G.R., and Jatlow, P.I. (1985). Increased anxiogenic effects of caffeine in panic disorders. *Arch. Gen. Psychol.* **42**:233–243.

Christmas, A.J., and Maxwell, D.R. (1970). A comparison of the effects of some benzodiazepines and other drugs on aggressive and exploratory behaviour in mice and rats. *Neuropharmacology* **9**:17–29.

Clement, Y., and Chapouthier, G. (1997). Biological bases of anxiety. *Neurosci. Biobehav. Res.* **22**:623–633.

Concas, A., Cuccheddu, T., Floris, S., Mascia, M.P., and Biggio, G. (1994). 2-Chloro-N6-cyclopentyladenosine (CCPA), and adenosine A_1 agonist, suppressed ethanol withdrawal syndrome in rats. *Alcohol Alcoholism* **29**:261–264.

Concas, A., Sanna, E., Mascia, M.P., Serra, M., and Biggio, G. (1990). Diazepam enhances bicuculline-induced increase of *t*-[^{35}S]butylbicyclophosphorothionate binding in unwashed membrane preparations from rat cerebral cortex. *Neurosci. Lett.* **112**:87–91.

Conceicao, I.M., Maiolini, M., Jr., Mattia, N., Vital, M.A., Santos, B.R., Smaili, S., and Frussa-Filho, R. (1992). Anxiety-induced antinociception in the mouse. *Braz. J. Med. Biol. Res.* **25**:831–834.

Conti, L.H., Maciver, C.R., Ferkany, J.W., and Abreu, M.I. (1990). Foot-shock-induced freezing behavior in rats as a model for assessing anxiolytics. *Psychopharmacology (Berlin)* **102**:492–497.

Cook, L., and Catania, A.C. (1964). Effects of drugs on avoidance and escape behavior. *Fed. Proc.* **23**:818–835.

Cook, L., and Kelleher, R.T. (1962). Drug effects on the behavior of animals. *Ann. NY Acad. Sci.* **96**:315–335.

Costall, B., Jones, B.J., Kelly, M.E., Naylor, R.J., and Tomkins, D.M. (1989). Exploration of mice in a black and white test box: Validation as a model of anxiety. *Pharmacol. Biochem. Behav.* **32**:777–785.

Cozzi, N.V., and Nichols, D.E. (1996). 5-HT_{2A} receptor antagonists inhibit potassium-stimulated γ-aminobutyric acid release in frontal cortex. *Eur. J. Pharmacol.* **309**:25–31.

Crawley, J.N. (1981). Neuropharmacologic specificity of a simple animal model for the behavioral actions of benzodiazepines. *Pharmacol. Biochem. Behav.* **15**:695–699.

Crawley, J.N. (1985). Exploratory behavior models of anxiety in mice. *Neurosci. Biobehav. Rev.* **9**:37–44.

Crawley, J.N., and Davis, L.G. (1982). Baseline exploratory activity predicts anxiolytic responsiveness to diazepam in five mouse strains. *Brain Res. Bull.* **8**:609–612.

Crawley, J.N., and Goodwin, F.K. (1980). Preliminary report of a simple animal behavior model for the anxiolytic effects of benzodiazepines. *Pharmacol. Biochem. Behav.* **13**:167–170.

Crawley, J.N., Blumstein, L.K., and Baldino, F. (1984a). Anxiolytic-like properties of fominoben. *Eur. J. Pharmacol.* **97**:227–281.

Crawley, J.N., Skolnick, F., and Paul, S.M. (1984b). Absence of intrinsic antagonist actions of benzodiazepine antagonists on an exploratory model of anxiety in the mouse. *Neuropharmacology* **23**:531–537.

Crawley, J.N., Marangos, P.J., Paul, S.M., Skolnick, P., and Goodwin, F.K. (1981). Interaction between purine and benzodiazepine: Inosine reverses diazepam-induced stimulation of mouse exploratory behavior. *Science* **211**:725–727.

Critchley, M.A.E., and Handley, S.L. (1987). Effects in the X-maze anxiety model of agents acting at 5-HT_1 and 5-HT_2 receptors. *Psychopharmacology* **93**:502–506.

Croiset, G., and DeWied, D. (1992). ACTH: A structure–activity study on pilocarpine-induced epilepsy. *Eur. J. Pharmacol.* **229**:211–216.

Crowley, J.J., Brodkin, E.S., Blendy, J.A., Berrettini, W.H., and Lucki, I. (2006). Pharmacogenomic evaluation of the antidepressant citalopram in the mouse tail suspension test. *Neuropsychopharmacology* **31**:2433–2442.

Cully, D.F., and Paress, P.S. (1991). Solubilization and characterization of a high-affinity ivermectin binding-site from *Caenorhabditis elegans Mol. Pharmacol.* **40**:326–332.

Cunha, J.M., and Masur, J. (1978). Evaluation of psychotropic drugs with a modified open field test. *Pharmacology* **16**:259–267.

Curle, P.F., Mocaer, E., Renard, P., and Guardiola, B. (1994). Anxiolytic properties of (+) S 20499, a novel serotonin 5-HT_{1A} full agonist, in the elevated plus-maze and social interaction tests. *Drug Dev. Res.* **32**:183–190.

Dekeyne, A., Brocco, M., Adhumeau, A., Gobert, A., and Millan, M.J. (2000). The selective serotonin (5-HT)$_{1A}$ receptor ligand, S15535, displays anxiolytic-like effects in the social interaction and Vogel models and suppresses dialysate levels of 5-HT in the dorsal hippocampus of freely-moving rats. *Psychopharmacology* **152**:55–66.

De Oliveira, R.W., and Guimaraes, F.S. (1999). Anxiolytic effect of methylene blue microinjected into the dorsal periaqueductal gray matter. *Brazil. J. Med. Biol. Res.* **32**:1529–1532.

Deren-Wesolek, A., Tatarczynska, E., and Chojnacka-Wojcik, E. (1998). The novel buspirone analogue, 8-[4-[2-(1,2,3,4-tetrahydroisoquinolinyl)[butyl]-8-azaspiro[4,5]decane-7,9-dione, with anxiolytic-like and antidepressant-like effects in rats. *J. Psychopharmacol.* **12**:380–384.

DeVane, C.L. (2000). Pharmacologic characteristics of ideal antidepressants in the 21st century. *J. Clin. Psychiatry* **61**:4–8.

Dews, P.B. (1964). A behavioral effect of amobarbital. *Naunyn Schmiedebergs Arch. Exp. Pathol. Pharmacol.* **248**:296–307.

Dews, P.B. (1955). Studies on behavior. I. Differential sensitivity to pentobarbital of pecking performance in pigeons depending on the schedule of reward. *J. Pharmacol. Exp. Ther.* **113**:393–401.

Dhonnchadha, B.A.N., Hascoet, M., Jolliet, P., and Bourin, M. (2003). Evidence for a 5-HT_{2A} receptor mode of action in the anxiolytic-like properties of DOI in mice. *Behav. Brain Res.* **147**:175–184.

Do-Rego, J.-C., Chatenet, D., Orta, M.-H., Naudin, B., Cudennec, C.L., Leprince, J., Scalbert, E., Vaudry, H., and Costentin, J. (2005). Behavioral effects of urotensin-II centrally administered in mice. *Psychopharmacology* **183**:103–117.

Dooley, D.J., and Klamt, I. (1993). Differential profile of the CCK_B receptor antagonist CI-988 and diazepam in the four-plate test. *Psychopharmacology* **112**:452–454.

Eckeli, A.L., Dach, F., and Rodrigues, A.L. (2000). Acute treatments with GMP produce antidepressant-like effects in mice. *Neuroreport* **11**:1839–1843.

Eguchi, J., Inomata, Y., and Saito, K.-I. (2001). The anxiolytic-like effect of MCI-225, a selective NA reuptake inhibitor with 5-HT_3 receptor antagonism. *Pharmacol. Biochem. Behav.* **68**:677–683.

Ehrensin, R.H., Kastin, A.J., Wurzlow, G.F., Michell, G.F., and Mebane, A.H. (1994). Improvement in major depression after low subcutaneous doses of MIF-1. *J. Affect. Disord.* **31**:227–233.

Ehrensin, R.H., and Kastin, A.J. (1974). Melanocyte-stimulating hormone-release inhibiting hormone as an antidepressant—Pilot-study. *Arch. Gen. Psychol.* **30**:63–65.

Engel, J.A., Egbe, P., Lijequist, S., and Soderpalm, B. (1989). Effects of amperozide in two animal models of anxiety. *Pharmacol. Toxicol.* **64**:429–433.

Eroglu, L., and Caglayan, B. (1997). Anxiolytic and antidepressant properties of methylene blue in animal models. *Pharmacol. Res.* **36**:381–385.

Fanselow, M.S. (1980). Conditional and unconditional components of post-shock freezing. *Pavlov. J. Biol. Sci.* 15:177–182.

Fanselow, M.S., and Helmstetter, F.J. (1988). Conditional analgesia, defensive freezing, and benzodiazepines. *Behav. Neurosci.* **102**:233–243.

Ferre, S., Von Euler, G., Johansson, J., Fredholm, B.B., and Fuxe, K. (1991). Stimulation of high-affinity adenosine-A2 receptors decreases the affinity of dopamine D2 receptors in rat striatal membranes. *Proc. Natl. Acad. Sci. USA* **88**:7238–7241.

Ferreira, V.M.M., and Morato, G.S. (1997). D-cycloserine blocks the effects of ethanol and HA-966 in rats tested in the elevated plus-maze. *Alcohol. Clin. Exp. Res.* **21**:1638–1642.

File, S.E. (1980). The use of social interaction as a method for detecting anxiolytic activity of chlordiazepoxide-like drugs. *J. Neurosci. Methods* **2**:219–238.

File, S.E., and Hyde, J.R.G. (1978). Can social interaction be used to measure anxiety? *Br. J. Pharmacol.* **62**:19–24.

File, S.E., Johnston, A.L., and Pellow, S. (1987). Effects of compounds acting at CNS 5-hydroxytryptamine systems on anxiety in the rat. *Br. J. Pharmacol.* **90**:265P.

Fishman, B.E., and Gianutsos, G. (1988). CNS biochemical and pharmacological effects of the isomers of hexachlorocyclohexane (lindane) in the mouse. *Toxicol. Appl. Pharmacol.* **93**:146–153.

Flood, J.F., Smith, G.E., and Cherkin, A. (1988). 2-Drug combinations of memory enhancers—Effect of dose ratio upon potency and therapeutic window in mice. *Life Sci.* **42**:2145–2154.

Flood, J.F., Smith, G.E., and Cherkin, A. (1985). Memory enhancement—Supra-additive effect of subcutaneous cholinergic drug-combinations in mice. *Psychopharmacology* **86**:61–67.

Frazer, A. (1997). Antidepressants. *J. Clin. Psychiatry* **58**:9–25.

Frenk, H. (1983). Pro- and anticonvulsant actions of morphine and the endogenous opioids: Involvement and interactions of multiple opiate and non-opiate systems. *Brain Res. Rev.* **6**:197–210.

Gale, K. (1985). Mechanisms of seizure control mediated by gamma-aminobutyric acid: A role of the substantia nigra. *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **44**:2414–2424.

Garant, D.S., Velisek, L., Sperber, E., and Moshe, S.L. (1992). Why do infants have seizures? *Int. Pediatr.* **7**:199–212.

Garant, D.S., Xu S.G., Sperber, E.F., and Moshe, S.L. (1995). Age-related differences in the effects of $GABA_A$ agonists microinjected into rat substantia nigra: Pro- and anticonvulsant actions. *Epilepsia* **36**:960–965.

Gardner, C.R., and Piper, D.C. (1982). Effects of agents which enhance GABA-mediated neurotransmission on licking conflict in rats and exploration in mice. *Eur. J. Pharmacol.* **83**:25–33.

Geller, I., and Seifter, J. (1960). The effects of meprobamate, barbiturate, D-amphetamine and promazine on experimentally induced conflict in the rat. *Psychopharmacologia* **1**:482–492.

Gonzalez, F.A., and Goldberg, S.R. (1977). Effects of cocaine and d-amphetamine on behavior maintained under various schedules of food presentation in squirrel monkeys. *J. Pharmacol. Exp. Ther.* **201**:33–43.

Goosens, K.A., and Maren, S. (2001). Contextual and auditory fear conditioning are mediated by the lateral, basal, and central amygdaloid nuclei in rats. *Learn. Mem.* **8**:148–155.

Graf, M., Kantor, S., Anheurer, Z.E., Modos, E.A., and Bagdy, G. (2003). M-CPP-induced self-grooming is mediated by 5-HT_{2C} receptors. *Behav. Brain Res.* **142**:175–179.

Griebel, G. (1995). 5-Hydroxytryptamine-interacting drugs in animal models of anxiety disorders: More than 30 years of research. *Pharmacol. Ther.* **65**:319–395.

Griebel, G., Belzung, C., Perrault, G., and Sanger, D.J. (2000a). Differences in anxiety-related behaviours and in sensitivity to diazepam in inbred and outbred strains of mice. *Psychopharmacology* **148**:164–170.

Griebel, G., Rodgers, R.J., Perrault, G., and Sanger, D.J. (2000b). The effects of compounds varying in selectivity as 5-HT_{1A} receptor antagonists in three rat models of anxiety. *Neuropharmacology* **39**:1848–1857.

Griebel, G., Simiand, J., Steinberg, R., Jung, M., Gully, D., Roger, P., Geslin, M., Scatton, B., Maffrand, J-P., and Soubrie, P. (2002a). 4-(2-Chloro-4-methoxy-5-methylphenyl)-*N*-[(1*S*)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]5-methyl-*N*-(2-propynyl)-1, 3-thiazol-2-amine hydrochloride (SSR125543A), a potent and selective corticotrophin-releasing factor1 receptor antagonist. II. Characterization in rodent models of stress-related disorders. *J. Pharmacol. Exp. Ther.* **301**:333–345.

Griebel, G., Simiand, J., Serradell-Le Gal, C., Wagnon, J., Pascal, M., Scatton, B., Maffrand, J.-P., and Soubrie, P. (2002b). Anxiolytic- and antidepressant-like effects of the non-peptide vasopressin V_{1b} receptor antagonist, SSR149415, suggest an innovative approach for the treatment of stress-related disorders. *Proc. Natl. Acad. Sci. USA* **99**:6370–6375.

Gudelsky, G.A., Koenig, J.I., and Meltzer, H.Y. (1988). Involvement of serotonin receptor subtypes in thermoregulatory responses. In:

5-HT Agonists as Psychoactive Drugs (eds. Rech, R.H., and Gudelsky, G.A.). NPP Books, Ann Arbor, MI, pp. 127–142.

Guimaraes, F.S., Carobrez, A.P., de Aguiar, J.C., and Graeff, F.G. (1991). Anxiolytic effect in the elevated plus-maze of the NMDA receptor antagonist-AP7 microinjected into the dorsal periaqueductal gray. *Psychopharmacology* **103**:91–94.

Guimaraes, F.S., de Aguiar, J.C., Del Bel, E.A., and Ballejo, G. (1994). Anxiolytic effect of nitric oxide synthase inhibitors microinjected into the dorsal central grey. *NeuroReport* **5**:1929–1932.

Hall, C.S. (1934). Emotional behavior in the rat: I. Defecation and urination as measures of individual differences in emotionality. *J. Comp. Psychol.* **18**:385–403.

Handley, S.L., and Mithani, S. (1984). Effects of alpha-adrenoceptor agonists and antagonists in a maze-exploration model of 'fear'-motivated behaviour. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **327**:1–5.

Hanson, H.M., Witoslawski, J.J., and Campbell, E.H. (1967). Drug effects in squirrel monkeys trained on a multiple schedule with a punishment contingency. *J. Expos. Anal. Behav.* **10**:565–569.

Hascoet, M., and Bourin, M. (1998). A new approach to the light/dark test procedure in mice. *Pharmacol. Biochem. Behav.* **60**:645–653.

Hascoet, M., Bourin, M., Colombel, M.C., Fiocco, A.J., and Baker, G.B. (2000). Anxiolytic-like effects of antidepressants after acute administration in a four-plate test in mice. *Pharmacol. Biochem. Behav.* **65**:339–344.

Hascoet, M., Bourin, M., and Nic Dhonnchadha, B.A. (2001). The mouse light–dark paradigm: A review. *Prog. Neuro-Psychopharmacol. Biol. Psychiat.*, **25**:141–166.

Hasenohrl, R.U., Jentjens, O., Silva, M.A.D.S., Tomaz, C., and Huston, J.P. (1998). Anxiolytic-like action of neurokinin substance P administered systemically or into the nucleus basalis magnocellularis region. *Eur. J. Pharmacol.* **354**:123–133.

Hashimoto, S., Inoue, T., and Koyama, T. (1996). Serotonin reuptake inhibitor reduce conditioned fear stress-induced freezing behavior in rats. *Psychopharmacology (Berlin)* **123**:182–186.

Hauser, C.A.E., Chesnoy-Marchais, D., Robel, P., and Beaulieu, E.E. (1995). Modulation of recombinant $\alpha 6\beta 2\gamma 2$ $GABA_A$ receptors by neuroactive steroids. *Eur. J. Pharmacol.* **289**:249–257.

Haut, S.R., Veliskova, J., and Moshe, S.L. (2004). Susceptibility of immature and adult brains to seizure effects. *Lancet Neurol.* **3**:608–617.

Hjorth, S., Engel, J.A., and Carlsson, A. (1986). Anticonflict effects of low doses of the dopamine agonist apomorphine in the rat. *Pharmacol. Biochem. Behav.* **24**:237–240.

Hjorth, S., Soderpalm, B., and Engel, J.A. (1987). Biphasic effect of L-5-HTP in the Vogel conflict model. *Psychopharmacology (Berlin)* **92**:96–99.

Hogg, S. (1996). A review of the validity and variability of the elevated plus-maze as an animal model of anxiety. *Pharmacol. Biochem. Behav.* **54**:21–30.

Homayoun, H., Khavandgar, S., and Dehpour, A.R. (2002). The role of α_2-adrenoceptors in the modulatory effects of morphine on seizure susceptibility in mice. *Epilepsia* **43**:797–804.

Honar, H., Riazi, K., Homayoun, H., Sadeghipour, H., Rashidi, N., Ebrahimkhani, M.R., Mirazi, N., and Dehpour, A.R. (2004). Ultra-low dose naltrexone potentiates the anticonvulsant effect of low dose morphine on clonic seizures. *Neuroscience* **129**:733–742.

Horvath, K., Andrasi, F., Botka, P., and Hamori, T. (1992). Anxiolytic profile of girisopam and GYKI 52 322 (EGIS 6775). Comparison with chlordiazepoxide and buspirone. *Acta Physiol. Hung.* **79**:153–161.

Horvath, E.J., Horvath, K., Hamori, T., Fekete, M.I.K., Solyom, S., and Palkovits, M. (2000). Anxiolytic 2,3-benzodiazepines, their specific binding to the basal ganglia. *Prog. Neurobiol.* **60**:309–342.

Howell, L.L., and Byrd, L.D. (1995). Serotonergic modulation of the behavioral effects of cocaine in the squirrel monkey. *J. Pharmacol. Exp. Ther.* **275**:1551–1559.

Howell, L.L., and Byrd, L.D. (1991). Characterization of the effects of cocaine and GBR 1209, a dopamine uptake inhibitor, on behavior in the squirrel monkey. *J. Pharmacol. Exp. Ther.* **258**:178–185.

Hu, R.Q., Koh, S., Torgerson, T., and Cole, A.J. (1998). Neuronal stress and injury in C57/BL mice after systemic kainic acid administration. *Brain Res.* **810**:229–240.

Huang, J., and Casida, J.E. (1997). Avermectin B_{1a} binds to high- and low-affinity sites with dual effects on the γ-aminobutyric acid-gated chloride channel of cultured cerebellar granule neurons. *J. Pharmacol. Exp. Ther.* **281**:261–266.

Hughes, R.N. (1972). Chlordiazepoxide modified exploration in rats. *Psychopharmacologia* **24**:462–469.

Im, H.K., Im, W.B., Pregenzer, J.F., Carter, D.B., Jacobsen, E.J., and Hamilton, B.J. (1995). Characterization of U-97775 as a $GABA_A$ receptor ligand of dual functionality in cloned rat $GABA_A$ receptor subtypes. *Brit. J. Pharmacol.* **115**:19–24.

Imaizumi, M., Miyazaki, S., and Onodera, K. (1994). Effects of xanthine derivatives in light/dark test in mice and the contribution of adenosine receptors. *Methods Find. Exp. Clin. Pharmacol.* **16**:639–644.

Inoue, T., Koyama, T., and Yamashita, I. (1992). From a standpoint of psychiatry: Effects of conditioned fear stress on monoaminergic systems in the rat brain. *Jpn. J. Clin. Pathol.*, **40**:227–233 (abstr. in English).

Ishida-Tokuda, K., Ohno, Y., Sakamoto, H., Ishibashi, T., Wakabayashi, J., Tojima, R., Morita, T., and Nakamura, M. (1996). Evaluation of perospirone (SM-9018), a novel serotonin-2 and dopamine-2 receptor antagonist, and other antipsychotics in the conditioned fear stress-induced freezing behavior model in rats. *Jpn. J. Pharmacol.* **72**:119–126.

Jacobsen, E.J., TenBrink, R.E., Stelzer, L.S., Belonga, K.L., Carter, D.B., Im, H.K., Im, W.B., Sethy, V.H., Tang, A.H., VonVoigtlander, P.F., and Petke, J.D. (1996a). High-affinity partial agonist imidazo[1,5-a]quinoxaline amides, carbamates, and ureas at the γ-aminobutyric acid A/benzodiazepine receptor complex. *J. Med. Chem.* **39**:158–175.

Jacobsen, E.J., Stelzer, L.S., Belonga, K.L., Carter, D.B., Im, W.B., Sethy, V.H., Tang, A.H., VonVoigtlander, P.F., and Petke, J.D. (1996b). 3-Phenyl-substituted imidazo[1,5-a]quinoxalin-4-ones and imidazo[1,5-a]quinoxaline ureas that have high affinity at the $GABA_A$/benzodiazepine receptor complex. *J. Med. Chem.* **39**:3820–3836.

Jacobsen, E.J., Stelzer, L.S., TenBrink, R.E., Belonga, K.L., Carter, D.B., Im, H.K., Im, W.B., Sethy, V.H., Tang, A.H., VonVoigtlander, P.F., Petke, J.D., Zhong, W.-Z., and Mickelson, J.W. (1999). Piperazine imidazo[1,5-a]quinoxaline ureas as high-affinity $GABA_A$ ligands of dual functionality. *J. Med. Chem.* **42**:1123–1144.

Jain, N., Kemp, N., Adeyemo, O., Buchanan, P., and Stone, T.W. (1995). Anxiolytic activity of adenosine receptor activation in mice. *Br. J. Pharmacol.* **116**:2127–2133.

Jones, B.J., Costall, B., Domeney, A.M., Kelly, M.E., Naylor, R.J., Oakely, N.R., and Tyers, M.B. (1988). The potential anxiolytic activity of GR38032F, a 5-HT_3-receptor antagonist. *Br. J. Pharmacol.* **93**:985–993.

Kademian, S.M.E., Bignante, A.E., Lardone, P., McEwen, B.S., and Volosin, M. (2005). Biphasic effects of adrenal steroids on learned helplessness behavior induced by inescapable shock. *Neuropsychopharmacology* **30**:58–66.

Kahn, R.S., van Praag, H.M., Wetzler, S., Asnis, G.M., and Barr, G. (1988). Serotonin and anxiety revisited. *Biol. Psychiatry* **23**:189–208.

Kamei, J., Ohsawa, M., Tsuji, M., Takeda, H., and Matsumiya, T. (2001). Modification of the effects of benzodiazepines on the exploratory behaviors of mice on a hole-board by diabetes. *Jpn. J. Pharmacol.* **86**:47–54.

Kaplan, G.B., Bharmal, N.H., Leite-Morris, K.A., and Adams, W.R. (1999). Role of adenosine A(1) and A(2A) receptors in the alcohol withdrawal syndrome. *Alcohol* **19**:157–162.

Kastin, A. J., Abel, D. A., Ehrensing, R. H., Coy, D. H., and Graf, M. V. (1984). Tyr–MIF-1 and MIF are active in the water wheel test for antidepressant drugs. *Pharm. Biochem. Behav.* **21**:767–771.

Katz, R.J., Roth, K.A., Carroll, B.J. (1981). Acute and chronic stress effects on open-field activity in the rat—Implications for a model of depression. *Neurosci. Biobehav. Rev.* **2**:247–251.

Kelleher, R.T., Fry, W., Deegan, J., and Cook, L. (1961). Effects of meprobamate on operant behavior in rats. *J. Pharmacol. Exp. Ther.* **133**:271–280.

Kilfoil, T., Michel, A., Montgomery, D., Whiting, R.L. (1989). Effects of anxiolytic and anxiogenic drugs on exploratory activity in a simple model of anxiety in mice. *Neuropharmacology* **28**:901–905.

Kim, M., Campeau, S., Falls, W.A., and Davis, M. (1993). Infusion of the non-NMDA receptor antagonist CNQX into the amygdala blocks the expression of fear-potentiated startle. *Behav. Neural. Biol.* **59**:5–8.

Klein, R.L., Sanna, E., McQuilkin, S.J., Whiting, P.J., and Harris, R.A. (1994). Effects of 5-HT_3 receptor antagonists on binding and function of mouse and human $GABA_A$ receptors. *Eur. J. Pharmacol.* **268**:237–246.

Kopf, S.R., Melani, A., Pedata, F., and Pepeu, G. (1999). Adenosine and memory storage: effect of A1 and A2 receptor antagonists. *Psychopharmacology* **146**:214–219.

Kralic, J.E., O'Buckley, T.K., Khisti, R.T., Hodge, C.W., Homanics, G.E., and Morrow, A.L. (2002). $GABA_A$ receptor alpha-1 subunit deletion alters receptor subtype assembly, pharmacological and behavioral responses to benzodiazepines and zolpidem. *Neuropharmacology* **43**:685–694.

Krogsgaard-Larsen, P., and Falch, E. (1981). GABA agonists: development and interactions with the $GABA_A$ receptor complex. *Mol. Cell Biochem.* **38**:129–146.

Kulkarni, S.K., and Reddy, D.S. (1996). Animal behavioral models for testing antianxiety agents. *Methods Find. Exp. Clin. Pharmacol.* **18**:219–230.

Kumar, S., and Sharma, A. (2005). Anti-anxiety activity studies on homeopathic formulations of *Turnera aphrodisiaca* ward. *ECAM* **2**:117–119.

Kuzmin, A., Semenova, S., Zvartau, E.E., Van Ree, J.M. (1996). Enhancement of morphine self-administration in drug naïve, inbred strains of mice by acute emotional stress. *Eur. Neuropsychopharmacol.* **6**:63–68.

Kuzmin, A., Zvartau, E., Gessa, G.L., Martellotta, M.C., and Fratta, W. (1992). Calcium antagonists isradipine and nimodipine suppress cocaine and morphine intravenous self-administration in drug-naïve mice. *Pharmacol. Biochem. Behav.* **41**:497–500.

Lader, M. (1982). Psychological effects of buspirone. *J. Clin. Psychiatry* **43**:62–68.

Lat, J. (1965). The spontaneous exploratory reactions as tool for psychopharmacological studies. *Second Int. Pharmacol. Meeting*. Oxford, Pergamon Press, **I**:47–66.

Laties, V.G., and Weiss, B. (1966). Influence of drugs on behavior controlled by internal and external stimuli. *J. Pharmacol. Exp. Ther.* **152**:388–396.

Lauretti, G.R., Ahmad, I., and Pleuvry, B.J. (1994). The activity of opioid analgesics in seizure models utilizing N-methyl-DL-aspartic acid, kainic acid, bicuculline and pentylenetetrazole. *Neuropharmacology* **33**:155–160.

Lee, C., and Rodgers, R.J. (1990). Antinociceptive effects of elevated plus-maze exposure: influence of opiate receptor manipulations. *Psychopharmacology* **102**:507–513.

Leung, W.C., Zheng, H., Law, S.L., and Xue, H. (2003). Anxiolytic-like action of orally administered *dl*-tetrahydropalmatine in elevated plus maze. *Prog. Neuron-Psychopharm. Biol. Psych.* **27**:775–779.

Levine, J., Kaplan, Z., Pettegrew, J.W., McClure, R.J., Gershon, S., Buriakovsky, I., and Cohen, H. (2005). Effect of intraperitoneal acetyl-L-carnitine (ALCAR) on anxiety-like behaviours in rats. *Int. J. Neuropsychopharmacol.* **8**:65–74.

Liljequist, S., and Tabakoff, B. (1986). Bicuculline-pentobarbital interactions on [S-35] TBPS binding in various brain-areas. *Life Sci.* **39**:851–855.

Liljequist, S., and Tabakoff, B. (1993). Bicuculline-produced regional differences in the modulation of S-35 TBPS binding by GABA, pentobarbital and diazepam in mouse cerebellum and cortex. *J. Pharmacol. Exp. Ther.* **264**:638–647.

Liu, Z., and Gershenfeld, H.K. (2003). An exploratory factor analysis of the tail suspension test in 12 inbred strains of mice and an F2 intercross. *Brain Res. Bull.* **60**:223–231.

Lloyd, G.K., Danielou, G., and Thuret, F. (1990). The activity of zolpidem and other hypnotics within the gamma-aminobutyric-acid (GABA-A) receptor supramolecular complex, as determined by S-35 tertbutylbicyclophosphorothionate (S-35-TERT-BPS) binding to rat cerebral-cortex membranes. *J. Pharmacol. Exp. Ther.* **255**:690–696.

Loke, W.H., Hinrichs, J.V., and Ghonheim, M.M. (1985). Caffeine and diazepam: Separate and combined effects on mood, memory and psychomotor performance. *Psychopharmacology* **87**:244–250.

Lucki, I., Rickels, K., Giesicke, M.A., and Geller, A. (1987). Differential effects of the anxiolytic drugs, diazepam and buspirone, on memory function. *Br. J. Clin. Pharmacol.* **23**:207–211.

Luparini, M.R., Garrone, B., Pazzagli, M., Pinza, M., and Pepeu, G. (2004). A cortical GABA-%-HT interaction in the mechanism of action of the antidepressant trazodone. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry.* **28**:1117–1127.

Majewska, M.D. (1987). Antagonist-type interaction of glucocorticoids with the GABA receptor-coupled chloride channel. *Brain Res.* **418**:377–382.

Majewska, M.D., and Schwartz, R.D. (1987). Pregnenolone-sulfate an endogenous antagonist of the γ-aminobutyric acid receptor complex in brain? *Brain Res.* **404**:355–360.

Majewska, M.D., Harrison, N.L., Schwartz, R.D., Barker, J.L., and Paul, S.M. (1986). Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor. *Science* **232**:1004–1007.

Maksay, G., and Simonyi, M. (1986). Kinetic regulation of convulsant (TBPS) binding by gabaergic agents. *Mol. Pharmacol.* **30**:321–328.

Maksay, G., and Van Rijn, C.M. (1993). Interconvertible kinetic states of T-butylbicycloorthobenzoate binding-sites of the gamma-aminobutyric acid(A) ionophores. *J. Neurochem.* **61**:2081–2088.

Marek, G.J., McDougle, C.J., Price, L.H., and Seiden, L.S. (1992). A comparison of trazodone and fluoxetine: Implications for a serotonergic mechanism of antidepressant action. *Psychopharmacology (Berlin)* **109**:2–11.

Maren, S., Aharonov, G., Stote, D.L., and Fanselow, M.S. (1996). *N*-Methyl-D-aspartate receptor in the basolateral amygdala are required for both acquisition and expression of conditional fear in rats. *Behav. Neurosci.* **110**:1365–1374.

Marriott, A.S., and Smith, E.F. (1972). An analysis of drug effects in mice exposed to a simple novel environment. *Psychopharmacologia (Berlin)* **24**:397–406.

Mathiasen, L., and Mirza, N.R. (2005). A comparison of chlordiazepoxide, bretazenil, L838,417 and zolpidem in a validated mouse Vogel conflict test. *Psychopharmacology* **182**:475–484.

Mayer, B., Brunner, F., and Schmidt, K. (1993). Inhibition of nitric oxide synthesis by methylene blue. *Biochem. Pharmacol.* **45**:367–374.

McArthur, R., and Borsini, F. (2006). Animal models of depression in drug discovery: A historical perspective. *Pharmacol. Biochem. Behav.* **84**:436–452.

McGurk, J.F., Bennet, M.V.L., and Zukin, R.S. (1990). Polyamines potentiate response of *N*-methyl-D-aspartate receptors expressed in *Xenopus* oocytes. *Proc. Natl. Acad. Sci. USA* **87**:9971–9974.

McMillan, D.E. (1973). Drugs and punished responding. I. Rate dependent effects under multiple schedules. *J. Expos. Anal. Behav.* **19**:133–145.

McMillan, D.E. (1969). Effects of d-amphetamine on performance under several parameters of multiple fixed-ratio, fixed-interval schedules. *J. Pharmacol. Exp. Ther.* **167**:26–33.

McMillan, D.E., and Campbell, R.J. (1970). Effects of *d*-amphetamine and chlordiazepoxide on spaced responding in pigeons. *J. Expos. Anal. Behav.* **14**:177–184.

McMillen, B.A., Matthews, R.T., Sanghera, M.K., Shepard, P.D., and German, D.C. (1983). Dopamine receptor antagonism by the novel antianxiety drug, buspirone. *J. Neurosci.* **3**:733–738.

Melchior, C.L., and Ritzmann, R.F. (1994a). Pregnenolone and pregnenolone sulfate, alone and with ethanol, in mice on the plus-maze. *Pharmacol. Biochem. Behav.* **48**:893–897.

Melchior, C.L., and Ritzmann, R.F. (1994b). Dehydroepiandrosterone is an anxiolytic in mice on the plus maze. *Pharmacol. Biochem. Behav.* **47**:437–441.

Metzenauer, P., Barnes, N.M., Costall, B., Gozlan, H., Hamon, M., Kelly, M.E., Murphy, D.A., and Naylor, R.J. (1992). Anxiolytic-like actions of anpirtoline in a mouse light-dark aversion paradigm. *NeuroReport* **3**:527–529.

Mi, X.J., Chen, S.W., Wang, W.J., Wang, R., Zhang, Y.J., Li, W.J., and Li, Y.L. (2005). Anxiolytic-like effect of paeonol in mice. *Pharmacol. Biochem. Behav.* **81**:683–687.

Mickelson, J.W., Jacobsen, E.J., Carter, D.B., Im, H.K., Im, W.B., Schreur, P.J.K.D., Sethy, V.H., Tang, A.H., McGee, J.E., and Petke, J.D. (1996). High-affinity α-aminoburyric acid A/benzodiazepine ligands: Synthesis and structure–activity relationship studies of a new series of tetracyclic imidazoquinoxalines. *J. Med. Chem.* **39**:4654–4666.

Millan, M.J., Brocco, M., Gobert, A., Dorey, G., Casara, P., and Dekeyne, A. (2001). Anxiolytic properties of the selective, non-peptidergic CRF_1 antagonists, CP154,526 and DMP695: A comparison to other classes of anxiolytic agent. *Neuropsychopharmacology* **25**:585–600.

Milman, A., Weizman, R., Rigai, T., Rice, K.C., and Pick, C.G. (2006). Behavioral effects of opioid subtypes compared with benzodiazepines in the staircase paradigm. *Behav. Brain Res.* **170**:141–147.

Molinengo, L., and Ricci-Gamalero, S. (1970). The "staircase maze" and the "simple staircase" in the analysis of the psychopharmacological action of CNS depressant. *Pharmacology* **4**:169–178.

Momose, K., Inui, A., Asakawa, A., Ueno, N., Nakajima, M., and Kasuga, M. (1998). Anxiolytic effect of motilin and reversal with GM-109, a motilin antagonist, in mice. *Peptides*, **19**:1739–1742.

Morse, W.H. (1962). Use of operant conditioning techniques for evaluating the effects of barbiturates on behavior. In: *The First Hahnemann Symposium on Psychosomatic Medicine*. Lea and Febiger, New York. pp. 275–281.

Moser, P.C. (1989). An evaluation of the elevated plus-maze test using the novel anxiolytic buspirone. *Psychopharmacology* **99**:48–53.

Moser, P.C., Tricklebank, M.D., Middlemiss, D.N., Mir, A.K., Hibert, M.F., and Fozard, J.R. (1990). Characterization of MDL 73005EF as a 5-HT_{1A} selective ligand and its effects in animal models of anxiety: Comparison with buspirone, 8-OH-DPAT and diazepam. *Brit. J. Pharmacol.* **99**:343–349.

Moshe, S.L., Brown, L.L., Kubova, H., Veliskova, J., Zukin, R.S., and Sperber, E.F. (1994). Maturation and segregation of brain networks that modify seizures. *Brain Res.* **665**:141–146.

Montgomery, K.C. (1958). The relation between fear induced by novel stimulation and exploratory behaviour. *J. Comp. Physiol. Psychol.* **48**:254–260.

N'Gouemo, P., Attia, M.B., and Belaidi, M. (1994). Effects of chloroquine on pentylenetetrazol-induced convulsions in mice. *Pharmacol. Res.* **30**:99–103.

Nishihara, I., Minami, T., Uda, R., Ito, S., Hyodo, M., and Hayaishi, O. (1995). Effect of NMDA receptor antagonists on prostaglandin E(2)-induced hyperalgesia in conscious mice. *Brain Res.* **677**:138–144.

Nolan, N.A., and Parkes, M.W. (1973). The effects of benzodiazepines on the behaviour of mice on a hole-board. *Psychopharmacologia (Berlin)*, **29**:277–288.

Nomura, S., Shimizu, J., Kinjo, M., Kametani, H., and Nakazawa, T. (1982). A new behavioral test for antidepressant drugs. *Eur. J. Pharmacol.* **83**:171–175.

Nunes-de-Souza, R.L., Canto-de-Souza, A., Da-Costa, M., Fornari, R.V., Graeff, F.G., and Pela, I.R. (2000). Anxiety-induced antinociception in mice: Effects of systemic and intra-amygdala

administration of 8-OH-DPAT and midazolam. *Psychopharmacology* **150**:300–310.

Obata, T., and Yamamura, H.I. (1986). The effect of benzodiazepines and β-carbolines on GABA-stimulated chloride influx by membrane vesicles from the rat cerebral cortex. *Biochem. Biophys. Res. Commun.* **141**:1–6.

Obata, T., Yamamura, H.I., Malatynska, E., Ikeda, M., Laird, H., Palmer, C.J., and Casida, J.E. (1988). Modulation of gamma-aminobutyric acid-stimulated chloride influx by bicycloorthocarboxylates, bicyclophosphorus esters, polychlorocycloalkanes and other cage convulsants. *J. Pharmacol. Exp. Ther.* **244**:802–806.

Ogawa, N., Hara, C., and Takaki, S. (1993). Anxiolytic activity of SC-48274 compared with those of buspirone and diazepam in experimental anxiety models. *Jpn. J. Pharmacol.* **61**:115–121.

Olivier, B., van Wijngaarden, I., and Soudijn, W. (2000). 5-HT_3 receptor antagonists and anxiety; a preclinical and clinical review. *Eur. Neuropsypharmacol.* **10**:71–95.

Oliveira, M.S., Furian, A.F., Royes, L.F.F., Fighera, M.R., Myskiw, J.C., Fiorenza, N.G., and Mello, C.F. (2004). Ascorbate modulates pentylenetetrazol-induced convulsions biphasically. *Neuroscience* **128**:721–728.

Olsen, R.W., and Snowman, A.M. (1982). Chloride-dependent enhancement by barbiturates of γ-aminobutyric acid receptor binding. *J. Neurochem.* **2**:1812–1823.

Olsen, R.W., and Snowman, A.M. (1983). [^{3}H]bicuculline methochloride binding to low-affinity γ-aminobutyric acid receptor sites. *J. Neurochem.* **41**:1653–1663.

Olsen, R.W., McCabe, R.T., and Wamsley, J.K. (1990). $GABA_A$ receptor subtypes: Autoradiographic comparison of GABA, benzodiazepine, and convulsant binding sites in the rat central nervous system. *J. Chem. Neuroanat.* **3**:59–76.

Onaivi, E.S., Green, M.R., and Martin, B.R. (1990). Pharmacological characterization of cannabinoids in the elevated plus maze. *J. Pharmacol. Exp. Ther.* **253**:1002–1009.

O'Neil, M.F., and Moore, N.A. (2003). Animal models of depression: Are there any? *Hum. Psychopharmacol.* **18**:239–254.

Palacios, J.M., Kuhar, M.J., Rapoport, S.I., and London, E.D. (1982). Effects of gamma-aminobutyric acid agonist and antagonist drugs on local cerebral glucose-utilization. *J. Neurosci.* **2**:853–860.

Papp, M., Vassout, A., and Gentsch, C. (2000). The NK-1 receptor antagonist NKP608 has an antidepressant-like effect in the chronic mild stress model of depression in rats. *Behav. Brain Res.* **115**:19–23.

Pelissier, T., Laurido, C., Hernandez, A., Constandil, L., and Eschalier, A. (2006). Biphasic effect of apomorphine on rat nociception and effect of dopamine D_2 receptor antagonists. *Eur. J. Pharmacol.* **546**:40–47.

Pellow, S., and File, S.E. (1986). Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze—A novel test of anxiety in the rat. *Pharmacol. Biochem. Behav.* **24**:525–529.

Pellow, S., Chopin, P., File, S.E., and Briley, M. (1985). Validation of open : closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *J. Neurosci. Methods* **14**:149–167.

Petit-Demouliere, B., Chenu, F., and Bourin, M. (2005). Forced swimming test in mice: A review of antidepressant activity. *Psychopharmacology* **177**:245–255.

Pich, E.M., and Samanin, R. (1986). Disinhibitory effects of buspirone and low doses of sulpiride and haloperidol in two experimental anxiety models in rats: Possible role of dopamine. *Psychopharmacology* **89**:125–130.

Pick, C.G., Peter, Y., Terkel, J., Gavish, M., and Weizman, R. (1996). Effect of the neuroactive steroid α-THDOC on staircase test behavior in mice. *Psychopharmacology* **128**:61–66.

Pick, C.G., Peter, Y., Paz, L., Schreiber, S., Gavish, M., and Weizman, R. (1997). Effect of the pregnane-related GABA-active steroid alphaxalone on mice performance in the staircase test. *Brain Res.* **765**:129–134.

Pignatiello, M.F., Olson, G.A.,Kastin, A.J., Ehrensing, R.H., McLean, J.H.,and Olson, R.D. (1989). MIF-1 is active in a chronic stress animal model of depression. *Pharmacol. Biochem. Behav.* **32**:737–742.

Plotnikoff, N.P., Kastin, A.J., Anderson, M.S., and Schally, A.V. (1971). DOPA potention by a hypothalamic factor, MSH release-inhibiting hormone (MIF). *Life Sci.* **10**:1279–1283.

Plotnikoff, N.P., Kastin, M.S., Anderson, M.S., and Schally, A.V. (1973). Deserpidine antagonism by a tripeptide, L-prolyl-L-leucyl-glycinamide. *Neuroendocrinology* **11**:67–71.

Pollard, G.T., and Howard, J.L. (1986). The staircase test: Some evidence of nonspecificity for anxiolytics. *Psychopharmacology* **89**:14–19.

Pomes, A., Rodriguezfarre, E., and Sunol, C. (1994a). Effects of organochlorine pesticides on Cl-36(-) flux in primary neuronal cultures. *Neurotoxicology* **15**:745–749.

Pomes, A., Rodriguezfarre, E., and Sunol, C. (1994b). Disruption of gaba-dependent chloride flux by cyclodienes and hexachlorocyclohexanes in primary cultures of cortical-neurons. *J. Pharmacol. Exp. Ther.* **271**:1616–1623.

Pomes, A., Frandsen, A., Sunol, C., Sanfeliu, C., Rodriguezfarre, E., and Schousboe, A. (1994c). Lindane cytotoxicity in cultured neocortical neurons is ameliorated by GABA and flunitrazepam. *J. Neurosci. Res.* **39**:663–668.

Porsolt, R.D., Le Pichon, M., and Jalfre, M. (1977). Depression: A new animal model sensitive to antidepressant treatments. *Nature* **266**:730–732.

Porsolt, R.D., Anton, G., Blavet, N. and Jalfre, M. (1978). Behavioural despair in rats: A new model sensitive to antidepressant treatments. *Eur. J. Pharmacol.* **47**:379–391.

Prediger, R.D.S., Batista, L.C., and Takahashi, R.N. (2004). Adenosine A_1 receptors modulate the anxiolytic-like effect of ethanol in the elevated plus-maze in mice. *Eur. J. Pharmacol.* **499**:147–154.

Pregenzer, J.F., Im, W.B., Carter, D.B., and Thomsen, D.R. (1993). Comparison of interaction of [^{3}H]muscimol, [^{35}S]*t*-butylbicyclophosphorothionate and [^{3}H]flunitrazepam with cloned γ-aminobutyric $acid_A$ receptors of the $\alpha1\beta2$ and $\alpha1\beta2\gamma2$ subtype. *Mol. Pharmacol.* **43**:801–806.

Prut, L., and Belzung, C. (2003). The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. *Eur. J. Pharmacol.* **463**:3–33.

Quock, R.M., Wetzel, P.J., Maillefer, R.H., Hodges, B.L., Curtis, B.A., and Czech, D.A. (1993). Benzodiazepine receptor-mediated behavioral effects of nitrous oxide in the rat social interaction test. *Pharmacol. Biochem. Behav.* **46**:161–165.

Reddy, D.S. (2004). Testosterone modulation of seizure susceptibility is mediated by neurosteroids 3α-androstanediol and 17β-estradiol. *Neuroscience* **129**:195–207.

Reddy, D.S., and Kulkarni, S.K. (1997). Differential anxiolytic effects of neurosteroids in the mirrored chamber behavior test in mice. *Brain Res.* **752**:61–71.

Reddy, D.S., Kaur, G., and Kulkarni, S.K. (1998). Sigma (sigma(1)) receptor mediated antidepressant-like effects of neurosteroids

in the Porsolt forced swim test. *Neuropharmacology* **9**:3069–3073.

Revel, L., Mannuni, L., Garofalo, P., and Makovec, F. (1998). CR 2945: A novel CCK_B receptor antagonist with anxiolytic-like activity. *Behav. Pharmacol.* **9**:183–194.

Rex, A., Voigt, J.P., Voits, M., and Fink, H. (1998). Pharmacological evaluation of a modified open-field test sensitive to anxiolytic drugs. *Pharmacol. Biochem. Behav.* **59**:677–683.

Riazi, K., Ronar, H., Homayoun, H., Rashidi, T., Dehghani, M., Sadeghipour, H., Gaskari, S.A., and Dehpour, A.R. (2004). Sex and estrus cycle differences in the modulatory effects of morphine on seizure susceptibility in mice. *Epilepsia* **45**:1035–1042.

Riblet, L.A., Taylor, D.P., Eison, M.S., and Stanton, H.C. (1982). Pharmacology and neurochemistry of buspirone. *J. Clin. Psychiatry* **43**:11–16.

Richelle, M., Xhenseval, B., Fontaine, O., and Thone, L. (1962). Action of chlordiazepoxide on two types of temporal conditioning in rats. *Int. J. Neuropharmacol.* **1**:381–391.

Ring, R.H., Malberg, J.E., Potestio, L., Ping, J., Boikess, S., Luo, B., Schechter,L.E., Rizzo, S., Rahman, Z., and Rosenzweig-Lipson, S. (2006). Anxiolytic-like activity of oxytocin in male mice: Behavioral and autonomic evidence, therapeutic implications. *Psychopharmacology* **185**:218–225.

Ripoll, N., David, D.J.P., Dailly, E., Hascoet, M., and Bourin, M. (2003). Antidepressant-like effects in various mice strains in the tail suspension test. *Behav. Brain Res.* **143**:193–200.

Ripoll, N., Hascoet, M., and Bourin, M. (2006). The four-plate test: Anxiolytic or analgesic paradigm? *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* **30**:873–880.

Risner, M.E., and Goldberg, S.R. (1983). A comparison of nicotine and cocaine self-administration in the dog: Fixed-ratio and progressive-ratio schedules of intravenous drug infusion. *J. Pharmacol. Exp. Ther.* **224**:319–326.

Rock, D.M., and Macdonald, R.L. (1995). Polyamine regulation of *N*-methyl-D-aspartate receptor channels. *Annu. Rev. Pharmacol. Toxicol.* **35**:463–482.

Rodgers, R.J. (1997). Animal model of 'anxiety': Where next? *Behav. Pharmacol.* **8**:477–496.

Rodgers, R.J., Cole, J.C., Aboualfa, K., and Stephenson, L.H. (1995). Ethopharmacological analysis of the effects of putative 'anxiogenic' agents in the mouse elevated plus-maze. *Pharmacol. Biochem. Behav.* **52**:805–813.

Ruarte, M.B., and Alvarez, E.O. (1999). Behavioral profiles displayed by rats in an elevated asymmetric plus-maze: Effects of diazepam. *Braz. J. Med. Biol. Res.* **32**:99–106.

Rubin, M.A., Berlese, D.B., Stiegemeier, J.A., Volkweis, M.A., Oliveira, D.M., dos Santos, T.L.B., Fenili, A.C., and Mello, C.F. (2004). Intra-amygdala administration of polyamines modulates fear conditioning in rats. *J. Neurosci.* **24**:2328–2334.

Rubin, M.A., Stiegemeier, J.A., Volkweis, M.A., Oliveira, D.M., Fenili, A.C., Boemo, R.L., Jurach, A., and Mello, C.F. (2001). Intra-amygdala spermidine administration improves inhibitory avoidance performance in rats. *Eur. J. Pharmacol.* **42**:35–39.

Rubin, M.A., Boemo, R.L., Jurach, A., Rojas, D.B., Zanolla, G.R., Obregon, A.D.C., Souza, D.O., and Mello, C.F. (2000). Intrahippocampal spermidine administration improves inhibitory avoidance performance in rats. *Behav. Pharmacol.* **11**:57–62.

Rutledge, C.O., and Kelleher, R.T. (1965). Interactions between the effects of methamphetamine and pentobarbital on operant behavior in the pigeon. *Psychopharmacologia* **7**:400–408.

Sakurada, T., Katsuyama, S., Sakurada, S., Inoue, M., Tan-No, K., Kisara, K., Sakurada, C., Ueda, H., and Sasaki, J. (1999). Nociceptin-induced scratching, biting and licking in mice: involvement of spinal NK1 receptors. *Br. J. Pharmacol.* **127**:1712–1718.

Sanchez, C. (2003). Stress-induced vocalization in adult animals. A valid model of anxiety? *Eur. J. Pharmacol.* **463**:133–143.

Sanchez, C., and Meier, E. (1997). Behavioral profiles of SSRIs in animal models of depression, anxiety and aggression. Are they all alike? *Psychopharmacology* **129**:197–205.

Sanger, D.J., and Blackman, D.E. (1975). The effects of tranquillizing drugs on timing behaviour in rats. *Psychopharmacologia* **44**:153–156.

Sanger, D.J., Key, M., and Blackman, D.E. (1974). Differential effects of chlordiazepoxide and *d*-amphetamine on responding maintained by a DRL schedule of reinforcement. *Psychopharmacologia* **38**:159–171.

Sapp, D.W., Witte, U., Turner, D.M., Longoni, B., Kokka, N., and Olsen, R.W. (1992). Regional variation in steroid anesthetic modulation of [^{35}S]TBPS binding to γ-aminobutyric $acid_A$ receptors in rat brain. *J. Pharmacol. Exp. Ther.* **262**:801–808.

Schreiber, R., and De Vry, J. (1993). Neuroanatomical basis for the antidepressant-like effects of the 5-HT(1A) receptor agonists 8-OH-DPAT and ipsapirone in the rat forced swimming test. *Behav. Pharmacol.* **4**:625–636.

Seiden, L.S., and Dykstra, L.A. (1977). *Psychopharmacology. A Biochemical and Behavioral Approach*. Van Nostrand Reinhold, New York.

Silvestre, J.S., Fernandez, A.G., and Palcios, J.M. (1996). Effects of 5-HT_4 receptor antagonists on rat behaviour in the elevated plus-maze test. *Eur. J. Pharmacol.* **309**:219–222.

Simiand, J., Keane, P.E., and Morre, M. (1984). The staircase test in mice: A simple and efficient procedure for primary screening of anxiolytic agents. *Psychopharmacology* **84**:48–53.

Simpson, R.E., O'Regan, M.H., Perkins, L.M., and Phillis, J.W. (1992). Excitatory transmitter amino acid release from the ischemic rat cerebral cortex: Effects of adenosine receptor agonists and antagonists. *J. Neurochem.* **58**:1683–1690.

Skolnick, P., Paul, S.M., and Weissman, B.A. (1984). Preclinical pharmacology of buspirone hydrochloride. *Pharmacotherapy* **4**:308–314.

Slotnick, B.M., and Jarvik, M.E. (1966). Deficits in passive avoidance and fear conditioning in mice with septal lesions. *Science* **154**:1207–1208.

Smith, S.S., Ruderman, Y., Gong, Q.H., and Gulinello, M. (2004). Effects of a low dose of ethanol in an animal model or premenstrual anxiety. *Alcohol* **33**:41–49.

Soderpalm, B., and Engel, J.A. (1988). Biphasic effects of clonidine on conflict behavior: Involvement of different alpha-adrenoceptors. *Pharmacol. Biochem. Behav.* **30**:471–477.

Soderpalm, B., Hjorth, S., and Engel, J.A. (1989). Effects of 5-HT_{1A} receptor agonists and L-5-HTP in Montgomery's Conflict Test. *Pharmacol. Biochem. Behav.* **32**:259–265.

Soubrie, P. (1986). Reconciling the role of central serotonin neurons in human and animal behavior. *Behav. Brain Sci.* **9**:319–364.

Soubrie, P., Thiebot, M.H., Simon, P., and Boissier, J.R. (1977). Effet des benzodiazepine sur les phenomenes d'inhibition qui controlent les compartements exploratoires et le recueil de l'information chez le rat. *J. Pharmacol. (Paris)*, **8**:393–403.

Spealman, R.D., Madras, B.K., and Bergman, J. (1989). Effects of cocaine and related drugs in nonhuman primates. II. Stimulant effects on schedule-controlled behavior. *J. Pharmacol. Exp. Ther.* **251**:142–149.

Spealman, R.D., Goldberg, S.R., Kelleher, R.T., Morse, W.H., Goldberg, D.M., Hakansson, C.G., Nieforth, K.A., and Lazer, E.S. (1979). Effects of norcocaine and some norcocaine derivatives on schedule-controlled behavior of pigeons and squirrel monkeys. *J. Pharmacol. Exper. Ther.* **210**:196–205.

Spealman, R.D., Goldberg, S.R., Kelleher, R.T., Goldberg, D.M., and Charlton, J.P. (1977). Some effects of cocaine and two cocaine analogs on schedule-controlled behavior of squirrel monkeys. *J. Pharmacol. Exp. Ther.* **202**:500–509.

Sperber, E.F., Wong, B.Y., and Wurpel, J.N.D., and Moshe, S.L. (1987). Nigral infusions of muscimol or bicuculline facilitate seizures in developing rats. *Dev. Brain Res.* **37**:243–250.

Sprosen, T., and Woodruff, G.N. (1990). Polyamines potentiate NMDA induced whole-cell currents in cultured striatal neurons. *Eur. J. Pharmacol.* **179**:477–478.

Squires, R.F., Casida, J.E., Richardson, M., and Saederup, E. (1983). [^{35}S]*t*-Butylbicyclophosphorothionate binds with high affinity to brain-specific sites coupled to γ-aminobutyric acid-A and ion recognition sites. *Mol. Pharmacol.* **23**:326–336.

Srinivasan, S., Sapp, D.W., Tobin, A.J., and Olsen, R.W. (1999). Biphasic modulation of GABA(A) receptor binding by steroids suggests functional correlates. *Neurochem. Res.* **24**:1363–1372.

Stefanski, R., Palejko, W., Bidzinski, A., Kostowski, W., and Plaznik, A. (1993). Serotonergic innervation of the hippocampus and nucleus accumbens septi and the anxiolytic-like action of midazolam and 5-HT$_{1A}$ receptor agonists. *Neuropharmacology* **32**:977–985.

Stephens, D.N., Meldrum, B.S., Weidmann, R., Schneider, C., and Grutzner, M. (1986). Does the excitatory amino-acid receptor antagonist 2-APH exhibit anxiolytic activity. *Psychopharmacology* **90**:166–169.

Steru, L., Chermat, R., Thierry, B., and Simon, P. (1985). The tail suspension test: A new method for screening antidepressants in mice. *Psychopharmacology* **85**:367–370.

Steru, L., Thierry, B., Chermat, R., Millet, B., Simon, P., and Porsolt, R.D. (1987). Comparing benzodiazepines using the staircase test in mice. *Psychopharmacology* **92**:106–109.

Stone, T.W., and Simmonds, H.A. (1991). *Purines: Basic and Clinical Aspects*. Kluwer Press, Dordrecht.

Subhash, M.N., Srinivas, B.N., and Vinod, K.Y. (2002). Alterations in 5-HT$_{1A}$ receptors and adenylyl cyclase response by trazodone in regions of rat brain. *Life Sci.* **71**:1599–1567.

Sullivan, R.M., Henke, P.G., and Ray, A. (1988). The effects of buspirone, a selective anxiolytic, on stress ulcer formation in rats. *Pharmacol. Biochem. Behav.* **31**:317–319.

Supavilai, P., and Karobath, M. (1984). [^{35}S]-*t*-Butylbicyclophosphorothionate binding sites are constitutents of the γ-aminobutyric acid-benzodiazepine receptor complex. *J. Neurosci.* **4**:1193–1200.

Takeda, H., Tsuji, M., and Matsumiya, T. (1998). Changes in head-dipping behavior in the hole-board test reflect the anxiogenic and/or anxiolytic state in mice. *Eur. J. Pharmacol.* **350**:21–29.

Taukulis, H.K., and Goggin, C.E. (1990). Diazepam-stress interactions in the rat: Effects on autoanalgesia and a plus-maze model of anxiety. *Behav. Neural. Biol.* **53**:205–216.

Teixeira, R.M., Santos, A.R.S., Ribeiro, S.J., Calixto, J.B., Rae, G.A., and De Lima, T.C.M. (1996). Effects of central administration of tachykinin receptor agonists and antagonists on plus-maze behavior in mice. *Eur. J. Pharmacol.* **311**:7–14.

Thiebot, M.H., Soubrie, P., Simon, P., and Boissier, J.R. (1973). Dissociation de deux composantes du comportement chez le rat sous l'effet de psychotropes. Application a l'etude des anxiolytiques. *Psychopharmacologia (Berlin)* **31**:77–90.

Thiebot, M.H., Soubrie, P., Simon, P., and Boissier, J.R. (1976). Specificite d'action des tranquillisants mineurs dans le test de l'escalier. Relation entre ces effets et leurs proprietes anxiolytiques. *J. Pharmacol. (Paris)* **7**:87–102.

Thierry, B., Steru, L., Simon, P., and Porsolt, R.D. (1986). The tail suspension test: ethical considerations. *Psychopharmacology* **90**:284–285.

Tissari, A.H., Stefanini, E., and Gessa, G.L. (1979). Stimulation of dopamine synthesis and activation of tyrosine hydroxylase by (-)sulpiride, sultopride and tiapride. In: *Sulpiride and Other Benzamides* (eds. Spano, P.F., Trabucchi, M., Corsini, G.U., and Gessa, G.L.). Italian Brain Research Foundation Press, Milan, pp. 3–9.

Treit, D. (1985). Animal models for the study of anti-anxiety agents: A review. *Neurosci. Biobehav. Rev.* **9**:203–222.

Turner, D.M., Ransom, R.W., Yang, J.S.-J., and Olsen, R.W. (1989). Steroid anesthetics and naturally occurring analogs modulate the γ-aminobutyric acid receptor complex at a site distinct from barbiturates. *J. Pharmacol. Exp. Ther.* **248**:960–966.

Uhde, T.W., Boulenger, J.P., Jimerson, D.C., and Post, R.M. (1984). Caffeine: Relationship to human anxiety, plasma MHPG and cortisol. *Psychopharmacol. Bull.* **20**:426–430.

Umezu, T. (1999). Effects of psychoactive drugs in the Vogel conflict test in mice. *Jpn. J. Pharmacol.* **80**:111–118.

Urban, J.H., Van de Kar, L.D., Lorens, S.A., and Bethea, C.L. (1986). Effect of the anxiolytic drugs buspirone on prolactin and corticotropine secretion in stressed and unstressed rats. *Pharmacol. Biochem. Behav.* **25**:457–462.

Vaidya, A.H., Rosenthal, D.I., Lang, W., Crooke, J.J., Benjamin, D., Ilyin, S.E., and Reitz, A.B. (2005). Oral buspirone causes a shift in the dose-response curve between the elevated-plus maze and Vogel conflict tests in Long-Evans rats: relation of brain levels of buspirone and 1-PP to anxiolytic action. *Methods Find. Exp. Clin. Pharmacol.* **27**:245–255.

Vanover, K.E., Piercey, M.F., and Woolverton, W.L. (1993). Evaluation of the reinforcing and discriminative stimulus effects of cocaine in combination with (+)-AJ 76 or clozapine. *J. Pharmacol. Exp. Ther.* **266**:780–789.

Varty, G.B., Morgan, C.A., Cohen-Williams, M.E., Coffin, V.L., and Carey, G.J. (2002a). The Gerbil elevated plus-maze I: behavioral characterization and pharmacological validation. *Neuropsychopharmacology* **27**:357–370.

Varty, G.B., Cohen-Williams, M.E., Morgan, C.A. et al. (2002b). The gerbil elevated plus-maze II. Anxiolytic-like effects of selective

neurokinin NK1 receptor antagonists. *Neuropsychopharmacology* **27**:371–379.

Vassout, A., Veenstra, S., Hauser, K., Ofner, S., Brugger, F., Schilling, W., and Gentsch, C. (2000). NKP608: a selective NK-1 receptor antagonist with anxiolytic-like effects in the social interaction and social exploration test in rats. *Regul. Peptides* **96**:7–16.

Velisek, L., Veliskova, J., Giorgi, F.S., and Moshe, S.L. (2006). Sex-specific control of flurothyl-induced tonic—clonic seizures by the substantia nigra pars reticulate during development. *Exp. Neurol.*, **201**:203–211.

Veliskova, J., and Moshe, S.L. (2001). Sexual dimorphism and developmental regulation of substantia nigra function. *Ann. Neurol.*, **50**:596–601.

Vogel, J.R., Beer, B., and Clody, D.E. (1971). A simple and reliable conflict procedure for testing anti-anxiety agents. *Psychopharmacologia* **21**:1–7.

Waller, M.B., and Morse, W.H. (1963). Effects of pentobarbital on fixed-ratio reinforcement. *J. Expos. Anal. Behav.* **6**:125–130.

Wedeking, P.W. (1974). Schedule-dependent differences among anti-anxiety drugs. *Pharmacol. Biochem. Behav.* **2**:465–472.

Wedeking, P.W. (1968). Stimulating effects of chlordiazepoxide in rats on a food reinforced FR schedule. *Psychonom. Sci.* **12**:31–32.

Wetzel, C.H.R., Vedder, H., Holsboer, F., Ziegigansberger, W., and Deisz, R.A. (1999). Bidirectional effects of the neuroactive steroid tetrahydrodeoxycorticosterone on GABA-activated Cl^- currents in cultured rat hypothalamic neurons. *Br. J. Pharmacol.* **127**:863–868.

Wiley, J.L., Compton, A.D., Holcomb, J.D., McCallum, S.E., Varvel, S.A., Porter, J.H., and Balster, R.L. (1998). Effects of modulation of NMDA neurotransmission on response rate and duration in a conflict procedure in rats. *Neuropharmacology* **37**:1527–1534.

Williams, K. (1997). Interactions of polyamines with ion channels. *Biochem. J.* **325**:289–297.

Williams, K., Romano, C., Dichter, M.A., and Molinoff, P.B. (1991). Modulation of the NMDA receptor by polyamines. *Life Sci.* **48**:469–498.

Williams, K., Dawson, V.L., Romano, C., Dichter, M.A., and Molinoff, P.B. (1990). Characterization of polyamines having agonist, antagonist, and inverse agonist effects at the polyamine recognition site of the NMDA receptor. *Neuron* **5**:199–208.

Wuttke, W., and Kelleher, R.T. (1970). Effects of some benzodiazepines on punished and unpunished behavior in the pigeon. *J. Pharmacol. Exp. Ther.* **172**:397–405.

Yokogawa, T., Kim, S.U., Krieger, C., and Puil, E. (2001). Analysis of GABA(A)- and GABA(B)-receptor mediated effects on intracellular Ca(2+) in DRG hybrid neurons. *Br. J. Pharmacol.* **134**:98–107.

Young, R., and Johnson, D.N. (1991). A fully automated light/dark apparatus useful for comparing anxiolytic agents. *Pharmacol. Biochem. Behav.* **40**:739–743.

Zaman, S.H., Shingai, R., Harvey, R.J., Darlison, M.G., and Barnard, E.A. (1992). Effects of subunit types of the recombinant $GABA_2$ receptor on the response to a neurosteroid. *Eur. J. Pharmacol.* **225**:321–330.

APPENDIX B

Psychopharmacology (2008) 201:115–123
DOI 10.1007/s00213-008-1252-2

ORIGINAL INVESTIGATION

Nesfatin-1 increases anxiety- and fear-related behaviors in the rat

Z. Merali · C. Cayer · P. Kent · H. Anisman

Received: 14 April 2008 / Accepted: 25 June 2008 / Published online: 1 August 2008

Abstract
Rationale Nesfatin-1, derived from the protein NEFA/nucleobindin2 (NUCB2), is a newly identified peptide that acts as a potent satiety agent. It has been reported that peptides involved in the regulation of ingestive behavior are also involved in the regulation of the stress response. However, the relation between nesfatin-1 and stressor-related behaviors like anxiety and/or fear has not yet been investigated.
Objective The effects of intracerebroventricular (ICV) injection of nesfatin-1 (0, 5, and 25 pmol/3 μl) were assessed in several paradigms that are thought to reflect anxiety and/or fear in rats.
Results Consistent with an anxiogenic effect, nesfatin-1 dose-dependently decreased the percentage of time spent on the open arms of the elevated plus maze, increased latency to approach, and decreased consumption of a palatable snack in an anxiogenic (unfamiliar) environment. Moreover, ICV nesfatin-1 increased the fear-potentiated startle response and the time spent freezing to both context and conditioned cues in a conditioned emotional response test.
Conclusions These findings suggest that in addition to its role as a satiety peptide, nesfatin-1 may also be involved in the mediation of anxiety- and/or fear-related responses.

Keywords Nesfatin-1 · Anxiety · Fear · Elevated plus maze · Novelty · Fear-potentiated startle · Conditioned emotional response

Z. Merali · C. Cayer · P. Kent
Department of Psychology, University of Ottawa,
Ottawa, ON, Canada, K1N 6N5

Z. Merali
Department of Psychiatry, University of Ottawa,
Ottawa, ON, Canada, K1N 6N5

Z. Merali
Department of Cellular and Molecular Medicine,
University of Ottawa,
Ottawa, ON, Canada, K1N 6N5

H. Anisman
Institute of Neuroscience, Carleton University,
Ottawa, ON, Canada, K1S 5B6

Z. Merali (✉) · C. Cayer · P. Kent · H. Anisman
University of Ottawa Institute of Mental Health Research,
1145 Carling Avenue, Room 5432 Research Tower,
Ottawa, ON, Canada, K1Z 7K4
e-mail: merali@uottawa.ca

Introduction

Nesfatin-1 is a newly identified 82 amino acid peptide derived from the larger precursor protein, NEFA/nucleobindin2 (NUCB2) (Oh et al. 2006). This peptide, which is highly conserved from mice to man, is abundantly expressed in several hypothalamic regions, including the paraventricular nucleus (PVN), supraoptic nucleus, arcuate nucleus, lateral nucleus, and the zona incerta (Oh et al. 2006; Brailoiu et al. 2007). Beyond the hypothalamus, nesfatin-1 has also been identified in several other brain regions, including the Edinger–Westphal nucleus, central nucleus of the amygdala (CeA), nucleus of the solitary tract, caudal raphé nucleus, locus coeruleus, periaqueductal gray matter, and the dorsal motor nucleus of the vagus (Brailoiu et al. 2007; Fort et al. 2007). Nesfatin-1 has been proposed as a novel satiety agent, stemming, in part, from its widespread distribution in hypothalamic regions involved in appetite and metabolic regulation (Oh et al. 2006; Brailoiu et al. 2007). Moreover, central administration of nesfatin-1 dose-dependently suppressed food intake, whereas intracerebroventricular (ICV) administration of antibodies directed against the peptide increased food intake (Oh et al. 2006).

It addition to its role in the regulation of feeding behavior, it is likely that this peptidergic system has functional consequences involving other physiological processes. In this regard, it has been suggested that, as behaviors associated with feeding are incompatible with defensive behaviors, those systems associated with reductions in consummatory behavior should be linked directly or indirectly to those subserving stressor reactivity (Akana et al. 1994; Dallman et al. 1995; Merali et al. 1998; Pecoraro et al. 2004). In support of this contention, it is not only well-documented that stressor exposure alters food intake (Levine and Morley 1981; Morley et al. 1983; Marti et al. 1994; Valles et al. 2000; Tamashiro et al. 2007), but that appetitive stimuli influence the stress response including the secretion of glucocorticoids (Follenius et al. 1982; Al-Damluji et al. 1987; Karbonits et al. 1996; Piazza and Le Moal 1997; Merali et al. 1998). Moreover, several investigators have reported that the state of hunger (fasting vs. satiated) as well as the type of meal consumed (high vs. low fat content) influences the responsiveness of the hypothalamic–pituitary–adrenal axis to stressor exposure (Shiraishi et al. 1984; Schwartz et al. 1995; Dallman et al. 1995; Leal and Moreira 1997; Tannenbaum et al. 1997). It is also noteworthy that many of the peptides involved in the central regulation of food intake also mediate or moderate the stress response. These peptides include (but are not limited to) corticotropin-releasing hormone (CRH), gastrin-releasing peptide, leptin, ghrelin, orexin, neuropeptide Y, and cholecystokinin (Crawley and Corwin 1994; Dallman et al. 1995; Hanson and Dallman 1995; Merali et al. 1998; Koob and Heinrichs 1999; Ahima and Flier 2000; Ueta et al. 2003; Spinazzi et al. 2006; Anisman et al. 2008).

In light of these observations, the primary objective of the present investigation was to assess the effects of central nesfatin-1 administration on stressor-related behaviors reflecting anxiety and/or fear. To this end, animals were centrally injected with one of two doses of nesfatin-1 (5 or 25 pmol; similar to the doses employed by Oh et al. 2006) and were assessed in several behavioral paradigms including those that measure innate (unlearned) anxiety responses (elevated plus maze [EPM] and novelty-induced suppression of food intake), and conditioned (learned) fear responses (fear-potentiated startle [FPS] and conditioned emotional response [CER]).

Materials and methods

Subjects

Male Sprague–Dawley rats from Charles River Laboratories (Saint-Constant, Quebec), weighing 275–300 g upon arrival, were doubly housed in standard plastic cages (45×25×20 cm) until surgery. In the housing/testing room, lighting was maintained on a 12-h light/dark cycle (lights on at 0700 hours) and the temperature (22°C) and humidity (63%) were kept constant. Throughout the study, including an initial 1-week period of acclimatization to the laboratory, animals had free access to food and water. All experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals as well as with the guidelines established by the Canadian Council on Animal Care. All experiments were approved by the Animal Care Committee of the University of Ottawa.

Surgery

Animals were anesthetized with halothane (2.5%) and stereotaxically implanted with a 22-gauge stainless steel guide cannula (Plastics One, Roanoke, VA, USA) aimed at the third ventricle. For pain control, the rats received oral Tylenol (100–200 mg/kg) for 3 days prior to and 3 days following surgery. In addition, they received rectal Tylenol (50 mg/kg) on the day of surgery and for 3 days post surgery. The placement coordinates (obtained from Paxinos and Watson 1986) were 4.4 mm posterior to bregma, 0 mm lateral, and 4.4 mm below the skull surface. The cannula was anchored to the skull with three stainless steel screws and dental cement. A stainless steel obturator (Plastics One, Roanoke, VA, USA) was inserted into the guide cannula and was kept patent by daily manipulation, which consisted of gently wrapping the rat in a cloth and rotating the obturator. Following surgery, animals were individually housed and allowed a 7-day recovery period prior to behavioral testing.

Drugs and injections

Nesfatin-1 (Phoenix Pharmaceuticals, Burlingame, CA, USA) was dissolved in Krebs–Ringer buffered saline solution (KRB) consisting of (in nmol): 2.7 K^+, 145 Na^+, 1.35 Ca^{2+}, 1.0 Mg^{2+}, 150 Cl^-, 0.05 ascorbate, pH 7.4 (Moghaddam and Bunney 1989). The control animals received an equivalent volume of KRB alone (vehicle). Nesfatin-1 or vehicle was microinjected into the third ventricle in a 3-μl volume, infused over 60 s, via an injection cannula (0.5 mm longer than the guide cannula) connected to an infusion pump with polyethylene tubing (Harvard Apparatus, Holliston, MA, USA). Following drug infusion, the injector was left in place for an additional 60 s to ensure drug diffusion.

Behavioral testing

Naïve animals were used in all experiments with the exception of the CER paradigm where the same rats were

used for both contextual and cued testing with 1 week intervening between contextual and cued tests. All testing was conducted under low illumination (30–40 lx) between 9:00 A.M. and 12:00 P.M. daily.

Elevated plus maze

The EPM, consisting of two open arms (50×10 cm) and two perpendicularly situated arms enclosed by 40 cm high walls, is commonly used to assess anxiety-like behavior in laboratory rodents (Pellow et al. 1985; File 1992). The EPM was elevated approximately 50 cm above the floor. A black curtain surrounded the chamber to limit the influence of spatial cues and other extraneous stimuli. A video camera was mounted above the arena to permit remote monitoring and recording.

Rats (n=8/group) were individually placed in the testing room for 1 h acclimatization and were then injected ICV with either vehicle (KRB solution) or nesfatin-1 (5 or 25 pmol) 15 min prior to testing. Each rat was then placed onto the open central platform of the EPM (facing a closed arm). The rats' behavior was monitored for 5 min and scored as follows: (1) frequency of entries into the open arms (all four paws on an open arm), (2) percentage of time spent on the open arms (time open/300×100), (3) frequency of entries in the closed arms, and (4) risk assessment behavior (unprotected head dips; head protruding over the edge of an open arm and down toward the floor). Between tests, the EPM was cleaned with 70% ethanol. The percent of time in the open arms, frequency of open arm entries, and unprotected head dips are all validated measures of anxiety-like behavior on the EPM (Pellow et al. 1985; File 1992; Griebel et al. 1997; Carobrez and Bertoglio 2005). Increases in these measures are indicative of reduced anxiety, whereas decreases suggest increased anxiety (File 1992). In contrast, the frequency of closed arm entries is an index of general activity (Cruz et al. 1994).

Approach to a palatable snack in a novel environment

When presented with a familiar snack in a novel (anxiogenic) environment, rodents are reluctant to approach and consume the treat. Anxiolytic agents have been shown to attenuate the reluctance to approach the snack and increase the amount of snack consumed, thus validating this model as a test of anxiety (Merali et al. 2003; Merali et al. 2004). Rats were habituated to a novel, highly palatable snack (Christie HoneyMaid© Graham Crumbs) presented in their home cage (15 min snack access) for eight consecutive days. The snack was presented in a 6 cm diameter ceramic dish placed in the center of the cage. During the last 3 days of this habituation period, when the approach and consumption parameters had stabilized (intake varied by less than ± 0.5 g), baseline measures of the latency to initiate consumption as well as the amount consumed were recorded. On the test day, rats received either ICV nesfatin-1 (5 or 25 pmol; n=8/group) or vehicle (KRB; n=10) and were then returned to their home cage. Fifteen minutes later, the rats were presented with the same (now familiar) palatable snack. The latency to initiate snack consumption and the amount consumed (over 15 min) were monitored. For the next 2 days, rats were again presented with the snack in their home cages in the absence of drug treatment. This served as a drug washout period and also ensured that a stable baseline was maintained and that no preexisting group differences were present prior to novel cage testing. On the following day, rats received either nesfatin-1 (5 or 25 pmol) or vehicle and were then returned to their home cage. Fifteen minutes following treatment, rats were transferred to a novel cage (freshly cleaned clear Plexiglas test cage without bedding) and presented with the familiar palatable snack. The latency to initiate snack consumption and the amount consumed (over 15 min) were monitored.

Fear-potentiated startle

The startle apparatus (Coulbourn Instruments, Whitehall, PA, USA) consisted of a sound attenuated chamber containing two calibrated platforms (18×10 cm) designed to measure the animal's startle response. Animals were placed in a Teflon cage (18.5×11 cm) positioned atop the platforms. The cage floor consisted of stainless steel rods (4 mm diameter spaced 1.8 cm apart) connected to shock generators (Coulbourn Instruments; H13–16). Force changes produced by the rats' startle response were measured by the startle sensor platform. The resultant voltage output from the platform transducer was digitized by an analog-to-digital converter card, interfaced with the computer, and recorded using data acquisition software (Coulbourn AASS v3.02). Startle amplitude was defined as the maximum peak to peak voltage that occurred during the first 200 ms after onset of the auditory startle stimulus. A high-frequency speaker, mounted (24 cm) above the platforms, generated white noise, while tones (startle stimulus) were generated by a Sonalert model tone generator (75 kHz; Coulbourn Instruments).

The training and testing for FPS spanned 4 days. On day 1, rats (n=8–11/group) were placed inside the startle chamber and exposed to random bursts of white noise (95, 110, and 115 db) for acclimatization and establishment of individual baseline startle amplitudes. On day 2, animals received a conditioning session where a tone (conditioning stimulus; CS) was paired with a shock (unconditioned stimulus; US). Specifically, a 1.0-mA, 0.5 s foot shock (US) was administered during the last 500 ms of the CS (a

4 s tone; 75 KHz). There were seven CS–US trials with an average of 1 min (randomized) intertrial intervals (ITI). Forty-eight hours later (day 4), rats received either ICV vehicle or nesfatin-1 (5 or 25 pmol) 15 min before testing for fear potentiation. Twenty trials of 110 db white noise bursts (random 1 min ITI) were followed by five trials of tones paired with noise bursts, and finally, five noise-alone trials. Cages were cleaned with 70% ethanol between testing of each animal. Rats that have learned to associate the CS (tone) with the US (foot shock) typically display a greater startle amplitude in the presence of the CS (Davis et al. 1993; Davis 1993). Administration of anxiolytic compounds decrease the FPS response in rodents (Davis 1993).

Conditioned emotional response

The conditioning chamber (Coulbourn Instruments, Whitehall, PA, USA) measured 31×25×30 cm. The front and back walls were made of clear Plexiglas and the two side walls were made of stainless steel panels. The floor comprised 16 stainless steel rods (2 mm diameter, 3 cm apart), connected to a constant current shock generator (Coulbourn Instruments; model H13–16). A Sonalert tone generator (Coulbourn Instruments; 75 kHz, low setting) was situated in the top rear panel and provided the conditioning auditory cue.

All subjects completed 1 day of training followed by a day of testing 24 h later. Training for contextual fear occurred 1 week after surgery, whereas cued fear training followed 2 weeks from surgery. During the contextual training phase, subjects were placed in the conditioning chamber where they received six foot shocks (1.0 mA; 1 s in duration) on a random schedule with an average ITI of 1 min. Cued fear training consisted of the delivery of six pairings of a 20 s tone with a 1.0-mA (1 s) foot shock in the conditioning chamber. The shock was delivered during the final second of the 20 s tone. Again, each trial was delivered at an average ITI of 1 min.

On the test days, rats (n=7–9/group) received ICV injections of vehicle or nesfatin-1 (5 or 25 pmol) 15 min before testing. Contextual fear was assessed over an 8 min period by placing rats in the conditioning chamber where they had previously been shocked. Freezing behavior, which was used as an index of conditioned fear, was timed using the software program ODlog (Macropod Software). Freezing was defined as the absence of movement excluding involuntary respiratory movements (Blanchard and Blanchard 1969). Trained experimenters blind to the drug condition conducted evaluations of freezing responses. To assess the CER in the cued condition, rats were transferred to a novel environment of similar dimensions, but visually and textually distinct from the training chamber. Specifically, black laminate covered the walls, and the floor was smooth (instead of a grid bar floor) and covered with bedding chips. Animals were allowed a 1 min exploration period and were then presented with the conditioned cue (the tone that had previously been paired with foot shock). A total of eight tones (each 20 s in duration) were presented at 1 min intervals (20 s tone+40 s ITI). Freezing was scored as described in the contextual test. Chambers were cleaned with 70% ethanol between each training and testing session.

Histology

Following completion of the experimental procedures, rats received an overdose of pentobarbital and 25% India ink (1 μl) was delivered through the injection cannula. Animals were then killed, and their brains were removed and frozen. The location of the cannula was verified histologically following thionin staining of the sections. With the exception of two cannulae, all others were correctly positioned.

Statistics

Data obtained from the EPM was analyzed using one-way analysis of variance (ANOVA) for each of the behavioral measures with treatment condition (vehicle, nesfatin-1 5 or 25 pmol) as the between-group factor. Data from approach to a snack in a novel environment experiment was analyzed using a mixed measures ANOVA with treatment condition (vehicle, nesfatin-1 5 and 25 pmol) as the between-group factor and test condition (home vs. novel cage) as the within-group factor. For the FPS experiment, to obtain an operational measure of fear, data was converted to percent change scores (mean startle amplitude on CS+noise trails− mean startle amplitude on noise-alone trials/mean startle amplitude on noise-alone trials×100) (see Walker and Davis 2002). The potentiated startle data was then analyzed using a one-way ANOVA with treatment condition as the between-group factor. For the CER data, raw freezing scores were transformed into a percentage of time spent freezing within each 1-min bin. These percentage scores were then averaged across the 8-min contextual and cued tests and analyzed using a one-way ANOVA with treatment condition as the between-group factor. In all experiments, follow-up analyses were conducted using t tests with a Bonferroni correction to protect the α at 0.05.

Results

Analyses of the EPM behaviors indicated that nesfatin-1 affected the percentage of time spent on the open arms ($F_{2,21}$=11.87; p<0.0003), the number of open arm entries

($F_{2,\ 21}$=5.07; p<0.015), and the number of unprotected head dips ($F_{2,\ 21}$=5.76; p<0.01)(see Fig. 1a–c). The follow-up tests revealed that rats that received nesfatin-1 spent significantly less time on the open arms (25 pmol), initiated significantly fewer open arm entries and unprotected head dips (25 pmol) relative to vehicle-treated rats. In contrast, nesfatin-1 administration had no effect on the number of closed arm entries (see Fig. 1d).

Figure 2a and b depict the effects of nesfatin-1 on the latency to approach the snack and the amount of a palatable snack consumed in both the home and novel cages. Mixed-measures ANOVA of the amount of snack consumed revealed a significant test condition (home vs. novel cage)×drug treatment (vehicle, nesfatin-1 5 or 25 pmol) interaction ($F_{2,\ 22}$=3.76; p<0.039). Follow-up tests of the simple effects comprising this interaction indicated that, in the home cage, there were no significant differences in the amount of snack consumed. However, a marked reduction in the amount of snack consumed was evident in the novel cage, which was significantly more pronounced following nesfatin-1 (5 or 25 pmol) administration. Similarly, analysis of the latency to consume the snack revealed a significant test condition (home vs. novel cage)×drug treatment (vehicle, nesfatin-1 5 or 25 pmol) interaction ($F_{2,\ 22}$=4.76; p<0.019). Follow-up tests again revealed that this interaction was attributable to a novelty-induced increase in latency to eat in the vehicle-treated animals tested in the novel cage and an even greater increase of the latency in animals treated with nesfatin-1 (5 and 25 pmol).

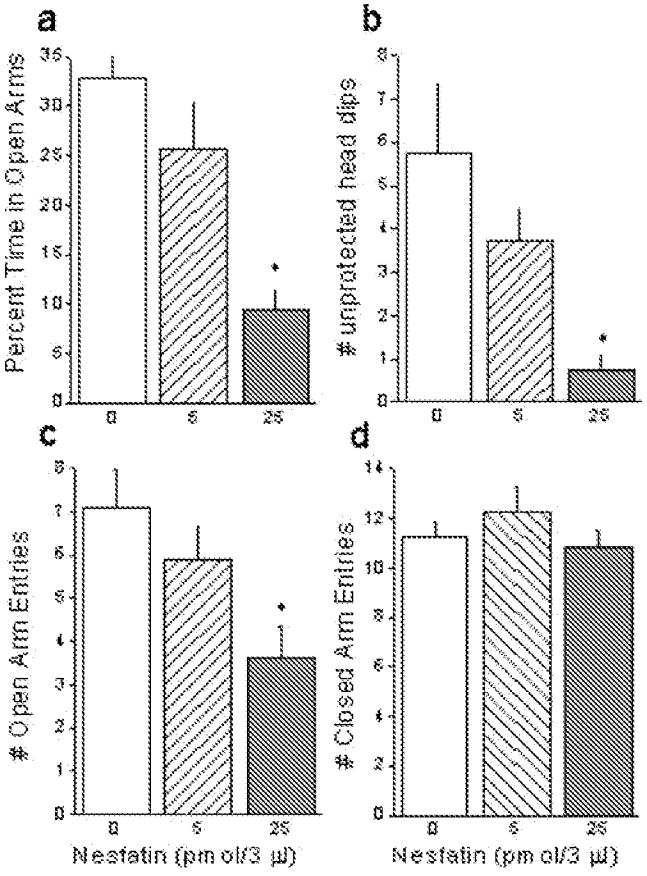

Fig. 1 Mean±SEM for several behaviors on the EPM among rats treated ICV with either vehicle (*open columns*), the low dose (5 pmol) of nesfatin-1 (*hatched columns*), or the high dose (25 pmol) of nesfatin-1 (*solid columns*). **a** Percent time (±SEM) animals spent on the open arms of the EPM, **b** the mean number of unprotected head dips (±SEM), **c** the mean number of times (±SEM) animals entered onto the open arms of the EPM, and **d** the mean number of times (±SEM) animals entered onto the closed arms of the EPM. *p<0.05; significantly different from vehicle condition

As depicted in Fig. 3, nesfatin-1 dose-dependently increased the expression of FPS ($F_{2,\ 26}$=4.01; p<0.03) without affecting the baseline startle amplitude (noise-alone trails; $F_{2,\ 26}$=1.19; p<0.32) (data not shown). The follow-up comparisons revealed that rats treated with the high dose of nesfatin-1 (25 pmol) displayed significantly greater startle potentiation than did the vehicle control group.

The effects of nesfatin-1 administration on freezing behavior in the contextual and cued CER tests are depicted in Fig. 4a and b. ANOVA revealed that, in both instances, percentage freezing varied as a function of the treatment condition ($F_{2,\ 20}$=3.42 and 3.99, ps<0.05). Follow-up comparisons indicated that, in both the contextual and cued tests, rats treated with the high dose of nesfatin-1 (25 pmol) exhibited more freezing behavior compared to vehicle-treated rats.

Discussion

In addition to its role in the regulation of feeding, the data of the present investigation suggest that nesfatin-1 is also involved in the mediation of anxiety- and/or fear-type behaviors. The EPM and novelty-induced suppression of food intake both measure behaviors reflecting unconditioned responses, and thus innate anxiety in rodents (Rodgers and Dalvi 1997; Merali et al. 2003). In the EPM, ICV administration of nesfatin-1 produced an anxiogenic effect reflected by significantly less time spent on the open arms of the maze and significantly fewer open arm entries and unprotected head dips (an index of risk assessment behavior). Importantly, the anxiogenic effects of nesfatin-1 on the EPM were not accompanied by nonspecific effects (changes of general locomotor activity) reflected by closed arm entries (File 1992; Cruz et al. 1994).

Central administration of nesfatin-1 also produced an anxiogenic effect in the approach to a snack in a novel environment paradigm. Typically, when a familiar snack is presented to a rat in a familiar environment, it will readily

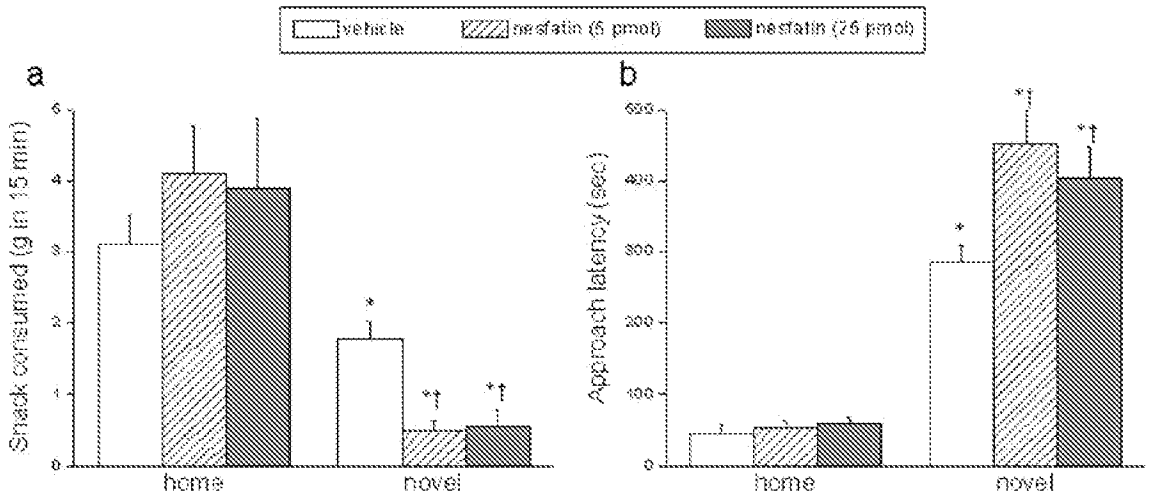

Fig. 2 Effect of nesfatin-1 on **a** snack consumption (in grams) and **b** on the latency to approach the snack (in seconds) in the home cage and novel cage conditions. Each column depicts snack consumption (mean±SEM over 15 min) or approach latency (mean±SEM) in the home cage or the novel cage conditions following ICV injection of vehicle (*open columns*), low-dose (5 pmol) nesfatin-1 (*hatched columns*), or high-dose (25 pmol) nesfatin-1 (*solid columns*). *$p<$ 0.05; significantly different from respective home cage baselines; †$p<$ 0.05; significantly different from condition matched control

approach and consume the snack. When the same snack is then presented in a novel environment, the latency to consume the snack is markedly increased and the amount of snack consumed is reduced, and these effects are antagonized by benzodiazepine administration (Merali et al. 2003; Merali et al. 2004). In the novel cage, central administration of nesfatin-1 at both doses (5 and 25 pmol) significantly increased the approach latency to the snack and decreased the amount of snack consumed relative to the vehicle-treated rats. In contrast, in the home cage, nesfatin-1 was without effect on either of these behavioral measures. This observation is interesting in light of the finding that central nesfatin-1 injection (4 and 20 pmol) suppresses food intake (Oh et al. 2006). Based on this finding, it might be tempting to speculate that, rather than playing a direct physiological role in appetite suppression, nesfatin-1 decreases food intake indirectly as a consequence of enhanced fear/anxiety. While this remains a possibility, it is important to consider that the effects of peptides on anxiety and satiety need not be mutually exclusive. In fact, as discussed earlier, many

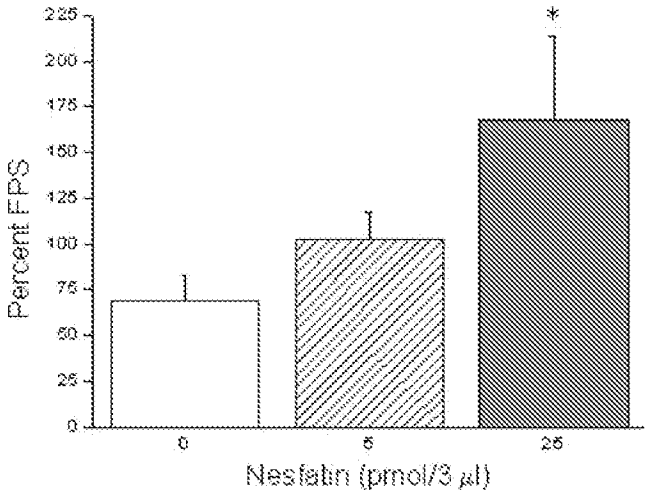

Fig. 3 FPS (mean±SEM) in rats treated ICV with either vehicle (*open columns*), low-dose (5 pmol) nesfatin-1 (*hatched columns*), or high-dose (25 pmol) nesfatin-1 (*solid columns*). *$p<0.05$; significantly different from vehicle condition

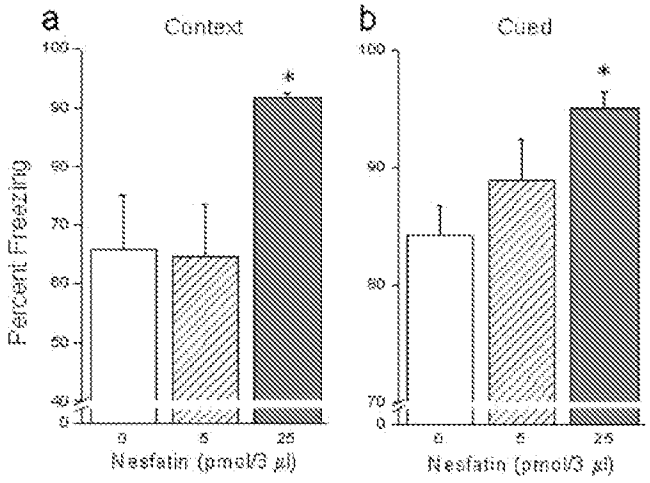

Fig. 4 Percentage of time engaged in freezing (mean±SEM) in the **a** contextual task and **b** in response to the fear cue applied in a different environment, following ICV administration of vehicle (*open columns*), the low dose (5 pmol) of nesfatin-1 (*hatched columns*), or the high dose (25 pmol) of nesfatin-1 (*solid columns*). *$p<0.05$; significantly different from vehicle condition

so-called satiety peptides also affect anxiety-like states. However, the fact that ICV infusion of either antiserum directed against nesfatin-1 or antisense oligonucleotide (which downregulated the mRNA for NUCB2) increased food intake and body weight gain suggests a physiological role of this peptide in food intake (Oh et al. 2006). This position is further supported by the observation that NUCB2 mRNA expression at regions known to be important in the regulation of food intake (PVN and supraoptic nucleus) is affected by the state of hunger (Oh et al. 2006; Kohno et al. 2008). Possible reasons why a decrease in food intake was not observed in the home cage may be attributable to differences in the paradigms employed. Specifically, rats in the Oh et al. study were fed normal rat chow as opposed to a palatable snack. Consistent with our present findings, we previously reported that central administration of CRH, a peptide that also has anxiogenic and anorectic properties, did not affect palatable snack consumption in the home cage (Merali et al. 2004). In contrast, however, central injection of another satiety peptide, gastrin-releasing peptide, reduced palatable snack consumption in the home cage (Merali et al. 2004). Although the neuronal mechanisms mediating these differences of peptide effects are not known, it appears that, at the doses used, the effect of nesfatin-1 was more aligned with the actions of CRH.

The effects of nesfatin-1 were also assessed in two models that measure learned (classically conditioned) fear responses, namely, the FPS and the CER paradigms (Davis 1990). In both models, central nesfatin-1 administration enhanced the fear response. In the FPS paradigm, rats treated with the high dose of nesfatin-1 (25 pmol) displayed an increased FPS response compared to vehicle-treated rats. This effect was apparent in the absence of any differences in startle reactivity in the noise-alone trials. Similarly, in both the contextual and cued components of the CER test, rats treated with nesfatin-1 (25 pmol) displayed significantly higher levels of freezing compared to vehicle-treated rats.

Taken together, these findings suggest a generalized role for nesfatin-1 in the mediation of anxiety- and fear-related behaviors, as the effects of the compound are evident across a variety of different anxiety- and fear-provoking situations. At present, the neuronal mechanism(s) mediating these effects are unknown. Based on recent findings, however, some speculations are possible. One obvious candidate might be through interactions with CRH. Indeed, the distribution patterns of these peptidergic systems, both abundantly found in hypothalamic regions as well as in extrahypothalamic sites such as the CeA (recognized as a key structure in the mediation of anxiety- and/or fear-related behaviors) (Davis 1992; Schulkin 2006), are in keeping with the contention that CRH and nesfatin-1 act in a collaborative or serial manner (Swanson et al. 1983; Oh et al. 2006; Brailoiu et al. 2007; Fort et al. 2007). In this regard, it has been reported that a subpopulation of nesfatin-1 neurons in the PVN coexpress CRH (Kohno et al. 2008; Price et al. 2008). It will be recalled that CRH is as a key mediator of the stress response, and like nesfatin-1, produces anxiogenic and fear-enhancing effects in several animal models of anxiety and/or fear (Kalin and Takahashi 1990; Heinrichs et al. 1995; Koob and Heinrichs 1999). In addition, CRH has clear anorectic properties, demonstrated by the ability of central CRH administration to suppress food intake (Morley and Levine 1982; Gosnell et al. 1983; Richard et al. 2002).

Other than CRH, another potential candidate through which nesfatin-1 may be mediating its anxiogenic effects is the melanocortin system. Supporting this contention, ICV administration of α-MSH (a melanocortin agonist) enhanced the expression of the gene encoding NUCB2 in the PVN (Oh et al. 2006), whereas administration of the melanocortin receptor antagonist, SHU9119, attenuated nesfatin-1-induced satiety (Oh et al. 2006). The melanocortin system appears to be involved in the mediation of anxiety-related behaviors, as administration of α-MSH reduced time spent on the open arms of the EPM as well as the number of licks in the Vogel test (Rao et al. 2003; Chaki et al. 2003). Moreover, pretreatment with SHU9119 dose-dependently attenuated stressor-induced anxiogenic behaviors (measured on the EPM) (Liu et al. 2007). There is also substantial evidence that the melanocortin system plays a role in feeding-related processes. For example, central administration of melanocortin agonists such as α-MSH or MTII reduce food intake (Rossi et al. 1998; Vergoni and Bertolini 2000). Interestingly, this effect was attenuated by blockade of CRH receptors suggesting a functional link between the CRH and central melanocortin systems (Dhillo et al. 2002; Lu et al. 2003). Taken together, these findings suggest that the relationship between nesfatin-1 and CRH and/or the melanocortin system might be interrelated. Of course, this suggestion is highly speculative, and further analyses will be needed to determine what role, if any, these systems play in the mediation of nesfatin-1-induced anxiogenic effects.

In summary, the present set of experiments demonstrates that central nesfatin-1 administration produces anxiogenic and fear-enhancing effects in several animal models that assess anxiety and/or fear. Like many other peptides that are involved in both the regulation of feeding behavior and stress-related responses, nesfatin-1 also appears to play a dual role in these physiological processes. Although the significance of this finding is not known, we previously suggested that peptidergic responses may be activated to draw attention to external events or cues of biological significance, irrespective of whether they involve food availability or a threat to survival (Merali et al. 1998).

Acknowledgements This study was supported by funds from the Canadian Institutes of Health Research (CIHR) and the Natural Science and Engineering Research Council of Canada (NSERC). HA is a Canadian Research Chair in Neuroscience. All experiments were conducted in accordance with the current laws of Canada. None of the authors have any direct or indirect conflicts of interests of a financial nature relevant to the present work.

References

Ahima RS, Flier JS (2000) Leptin. Annu Rev Physiol 62:413–437

Akana SF, Strack AM, Hanson ES, Dallman MF (1994) Regulation of activity in the hypothalamic–pituitary–adrenal axis is integral to a larger hypothalamic system that determines caloric flow. Endocrinology 135:1125–1134

Al-Damluji S, Iverson T, Thomas JM, Pendlebury DJ, Rees LH, Besser GM (1987) Food-induced cortisol secretion is mediated by central alpha-1 adrenoceptor modulation of pituitary ACTH secretion. Clin Endocrinol 26:629–636

Anisman H, Merali Z, Hayley S (2008) Neurotransmitter, peptides and cytokine processes in relation to depressive disorder: comorbidity between depression and neurodegenerative disorders. Prog Neurobiol 85:1–74

Blanchard RJ, Blanchard DC (1969) Crouching as an index of fear. J Comp Physiol Psychol 67:370–375

Brailoiu GC, Dun SL, Brailoiu E, Inan S, Yang J, Chang JK, Dun NJ (2007) Nesfatin-1: distribution and interaction with a G protein-coupled receptor in the rat brain. Endocrinology 148:5088–5094

Carobrez AP, Bertoglio LJ (2005) Ethological and temporal analyses of anxiety-like behavior: the elevated plus-maze model 20 years on. Neurosci Biobehav Rev 29:1193–1205

Chaki S, Ogawa S, Toda Y, Funakoshi T, Okuyama S (2003) Involvement of the melanocortin MC4 receptor in stress-related behavior in rodents. Eur J Pharmacol 474:95–101

Crawley JN, Corwin RL (1994) Biological actions of cholecystokinin. Peptides 15:731–755

Cruz APM, Frei F, Graeff FG (1994) Ethopharmacological analysis of rat behavior on the elevated plus-maze. Pharmacol Biochem Behav 49:171–176

Dallman MF, Akana SF, Strack AM, Hanson S, Sebastian RJ (1995) The neural network that regulates energy balance is responsive to glucocorticoids and insulin and also regulates HPA axis responsivity at sites proximal to CRF neurons. Ann NY Acad Sci 771:730–742

Davis M (1990) Animal models of anxiety based on classical conditioning: the conditioned emotional response (CER) and the fear-potentiated startle effect. Pharmacol Ther 47:147–165

Davis M (1992) The role of the amygdala in fear and anxiety. Annu Rev Neurosci 15:353–375

Davis M (1993) Pharmacological analysis of fear-potentiated startle. Braz J Med Biol Res 26:235–260

Davis M, Falls WA, Campeau S, Kim M (1993) Fear-potentiated startle: a neural and pharmacological analysis. Behav Brain Res 58:175–198

Dhillo WS, Small CJ, Seal LJ, Kim MS, Stanley SA, Murphy KG, Ghatei MA, Bloom SR (2002) The hypothalamic melanocortin system stimulates the hypothalamo-pituitary-adrenal axis in vitro and in vivo in male rats. Neuroendocrinology 75:209–216

File SE (1992) Behavioural detection of anxiolytic action. In: Elliot JM, Heal DJ, Marsden CA (eds) Experimental approaches to anxiety and depression. Wiley, Chichester, pp 25–44

Follenius M, Brandenberger G, Hietter B (1982) Diurnal cortisol peaks and their relationships to meals. J Clin Endocrinol Metab 55:757–761

Fort P, Butaud C, Salvert D, Shimizu H, Hashimoto K, Mori M, Luppi PH (2007) The satiety molecule nesfatin-1 is co-localized with MCH in hypothalamic neurons expressing Fos during paradoxical sleep in rats. Société des Neuroscience (8e Colloque Montpellier) Abstract:B.13

Gosnell BA, Morley JE, Levine AS (1983) A comparison of the effects of corticotropin releasing factor and sauvagine on food intake. Pharmacol Biochem Behav 19:771–775

Griebel G, Rodgers RJ, Perrault G, Sanger DJ (1997) Risk assessment behaviour: evaluation of utility in the study of 5-HT-related drugs in the rat elevated plus-maze test. Pharmacol Biochem Behav 57:817–827

Hanson ES, Dallman MF (1995) Neuropeptide Y (NPY) may integrate responses of hypothalamic feeding systems and the hypothalamic–pituitary–adrenal axis. J Neuroendocrinol 7:273–279

Heinrichs SC, Menzaghi F, Pich EM, Britton KT, Koob GF (1995) The role of CRF in behavioral aspects of stress. Ann NY Acad Sci 771:92–104

Kalin NH, Takahashi LK (1990) Fear-motivated behavior induced by prior shock experience is mediated by corticotropin-releasing hormone systems. Brain Res 509:80–84

Karbonits M, Trainer PJ, Nelson ML, Howse I, Kopelman PG, Besser GM, Grossman AB, Svec F (1996) Differential stimulation of cortisol and dehydroepiandrosterone levels by food in obese and normal subjects: relation to body fat distribution. Clin Endocrinol 45:699–706

Kohno D, Nakata M, Maejima Y, Shimizu H, Sedbazar U, Yoshida N, Dezaki K, Onaka T, Mori M, Yada T (2008) Nesfatin-1 neurons in paraventricular and supraoptic nuclei of the rat hypothalamus coexpress oxytocin and vasopressin and are activated by refeeding. Endocrinology 149:1295–1301

Koob GF, Heinrichs SC (1999) A role for corticotropin releasing factor and urocortin in behavioral responses to stressors. Brain Res 848:141–152

Leal AM, Moreira AC (1997) Food and the circadian activity of the hypothalamic–pituitary–adrenal axis. Braz J Med Biol Res 30:1391–1405

Levine AS, Morley JE (1981) Stress-induced eating in rats. Am J Physiol 241:R72–R76

Liu J, Garza JC, Truong HV, Henschel J, Zhang W, Lu XY (2007) The melanocortinergic pathway is rapidly recruited by emotional stress and contributes to stress-induced anorexia and anxiety-like behavior. Endocrinology 148:5531–5540

Lu XY, Barsh GS, Akil H, Watson SJ (2003) Interaction between alpha-melanocyte-stimulating hormone and corticotropin-releasing hormone in the regulation of feeding and hypothalamo-pituitary-adrenal responses. J Neurosci 23:7863–7872

Marti O, Marti J, Armario A (1994) Effects of chronic stress on food intake in rats: influence of stressor intensity and duration of daily exposure. Physiol Behav 55:747–753

Merali Z, McIntosh J, Kent P, Michaud D, Anisman H (1998) Aversive and appetitive events evoke the release of corticotropin-releasing hormone and bombesin-like peptides at the central nucleus of the amygdala. J Neurosci 18:4758–4766

Merali Z, Levac C, Anisman H (2003) Validation of a simple, ethologically relevant paradigm for assessing anxiety in mice. Biol Psychiatry 54:552–565

Merali Z, Khan S, Michaud DS, Shippy SA, Anisman H (2004) Does amygdaloid corticotropin-releasing hormone (CRH) mediate anxiety-like behaviors? Dissociation of anxiogenic effects and CRH release. Eur J Neurosci 20:229–239

Moghaddam B, Bunney BS (1989) Ionic composition of microdialysis perfusing solution alters the pharmacological responsiveness and basal outflow of striatal dopamine. J Neurochem 53:652–654

Morley JE, Levine AS (1982) Corticotropin-releasing factor, grooming and ingestive behavior. Life Sci 31:1459–1464

Morley JE, Levine AS, Rowland NE (1983) Minireview. Stress induced eating. Life Sci 32:2169–2182

Oh I, Shimizu H, Satoh T, Okada S, Adachi S, Inoue K, Eguchi H, Yamamoto M, Imaki T, Hashimoto K, Tsuchiya T, Monden T, Horiguchi K, Yamada M, Mori M (2006) Identification of nesfatin-1 as a satiety molecule in the hypothalamus. Nature 443:709–712

Paxinos G, Watson C (1986) The rat brain in stereotaxic coordinates. Academic Press, New York

Pecoraro N, Reyes F, Gomez F, Bhargava A, Dallman MF (2004) Chronic stress promotes palatable feeding, which reduces signs of stress: feedforward and feedback effects of chronic stress. Endocrinology 145:3754–3762

Pellow S, Chopin P, File SE, Briley M (1985) Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods 14:149–167

Piazza PV, Le Moal M (1997) Glucocorticoids as a biological substrate of reward: physiological and pathophysiological implications. Brain Res Rev 25:359–372

Price CJ, Hoyda TD, Samson WK, Ferguson AV (2008) Nesfatin-1 influences the excitability of paraventricular nucleus neurons. J Neuroendocrinol 20:245–250

Rao TL, Kokare DM, Sarkar S, Khisti RT, Chopde CT, Subhedar N (2003) GABAergic agents prevent alpha-melanocyte stimulating hormone induced anxiety and anorexia in rats. Pharmacol Biochem Behav 76:417–423

Richard D, Lin Q, Timofeeva E (2002) The corticotropin-releasing factor family of peptides and CRF receptors: their roles in the regulation of energy balance. Eur J Pharmacol 440:189–197

Rodgers RJ, Dalvi A (1997) Anxiety, defense and the elevated plus-maze. Neurosci Biobehav Rev 21:801–810

Rossi M, Kim MS, Morgan DG, Small CJ, Edwards CM, Sunter D, Abusnana S, Goldstone AP, Russell SH, Stanley SA, Smith DM, Yagaloff K, Ghatei MA, Bloom SR (1998) A C-terminal fragment of Agouti-related protein increases feeding and antagonizes the effect of alpha-melanocyte stimulating hormone in vivo. Endocrinology 139:4428–4431

Schulkin J (2006) Angst and the amygdala. Dialogues in Clinical Neuroscience 8:407–416

Schwartz MW, Dallman MF, Woods SC (1995) Hypothalamic response to starvation: implications for the study of wasting disorders. Am J Physiol 269:R949–R957

Shiraishi I, Honma K, Honma S, Hiroshige T (1984) Ethosecretogram: relation of behavior to plasma corticosterone in freely moving rats. Am J Physiol 247:R40–R45

Spinazzi R, Andreis PG, Rossi GP, Nussdorfer GG (2006) Orexins in the regulation of the hypothalamic–pituitary–adrenal axis. Pharmacol Rev 58:46–57

Swanson LW, Sawchenko PE, Rivier J, Vale WW (1983) Organization of ovine corticotropin-releasing factor (CRF)-immunoreactive cells and fibers in the rat brain: an immunohistochemical study. Neuroendocrinology 36:165–186

Tamashiro KL, Hegeman MA, Nguyen MM, Melhorn SJ, Ma LY, Woods SC, Sakai RR (2007) Dynamic body weight and body composition changes in response to subordination stress. Physiol Behav 91:440–448

Tannenbaum BM, Brindley DN, Tannenbaum GS, Dallman MF, McArthur MD, Meaney MJ (1997) High-fat feeding alters both basal and stress-induced hypothalamic–pituitary–adrenal activity in the rat. Am J Physiol 273:E1168–E1177

Ueta Y, Ozaki Y, Saito J, Onaka T (2003) Involvement of novel feeding-related peptides in neuroendocrine response to stress. Exp Biol Med 228:1168–1174

Valles A, Marti O, Garcia A, Armario A (2000) Single exposure to stressors causes long-lasting, stress-dependent reduction of food intake in rats. Am J Physiol 279:R1138–R1144

Vergoni AV, Bertolini A (2000) Role of melanocortins in the central control of feeding. Eur J Pharmacol 405:25–32

Walker DL, Davis M (2002) Quantifying fear potentiated startle using absolute versus proportional increase scoring methods: implications for the neurocircuitry of fear and anxiety. Psychopharmacology 164:318–328

Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
European Journal of Pharmacology 463 (2003) 3–33
ejp
www.elsevier.com/locate/ejphar

The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review

Laetitia Prut, Catherine Belzung*

EA3248, Psychobiologie des Émotions, Faculte des Sciences et Techniques, Universite Francois Rabelias, Parc de Grandmont Avenue Monge, 37200 Tours, France

Accepted 10 December 2002

Abstract

The open field is a very popular animal model of anxiety-like behavior. An overview of the literature on the action elicited by effective or putative anxiolytics in animal subjected to this procedure indicates that classical treatments such as benzodiazepine receptor full agonists or 5-HT_{1A} receptor full or partial agonists elicit an anxiolytic-like effect in this procedure in most cases (approximately 2/3). However, compounds (triazolobenzodiazepines such as adinazolam and alprazolam, selective serotonin reuptake inhibitors) that have a different spectrum of therapeutic efficacy in anxiety disorders such as panic attacks, generalized anxiety disorder or obsessive-compulsive disorder were poorly effective as anxiolytics in the open field test, suggesting that this paradigm may not model features of anxiety disorders. The procedure is also relevant for the study of compounds endowed with anxiogenic effects, as such effects were detected after treatments with benzodiazepine receptor inverse agonists or with corticotropin releasing factor (CRF) receptor agonists.

Keywords: Open field; Benzodiazepine; 5-HT (5-hydroxytryptamine: serotonin); Neuropeptide; Anxiety

1. Introduction

Hall (1934) originally described the open field test for the study of emotionality in rats. The procedure consists of subjecting an animal, usually a rodent, to an unknown environment from which escape is prevented by surrounding walls (Walsh and Cummins, 1976). Hall's apparatus consisted of a brightly illuminated circular arena of about 1.2 m diameter closed by a wall 0.45 m high. He placed rats individually in the outer ring of the open field and observed the rat's behavior for 2 min, during daily repeated trials. Rats were sometimes tested after 24 or 48 h food deprivation. Hall observed that rats walked more when they were food deprived, but not all rats ate. Animals that did not eat were termed emotional. When compared to non-emotional rats, they had fewer entries in the central part of the arena and higher levels of defecation.

The open field test is now one of the most popular procedure in animal psychology (see Belzung, 1999 for a review). Different versions are available, differing in shape of the environment (circular, square or rectangular), lighting (lighting from above with a bulb above the open field or lighting from underneath with a bulb placed under a transparent floor, sometimes red light is used), presence of objects within the arena such as platforms, columns, tunnels (see for example Takahashi and Kalin, 1989), etc. The procedure generally usually involves forced confrontation of a rodent with the situation. The animal is placed in the center or close to the walls of the apparatus and the following behavioral items are recorded for a period ranging from 2 to 20 min (usually 5 min): horizontal locomotion (number of crossings of the lines marked on the floor), frequency of rearing or leaning (sometimes termed vertical activity), grooming (protracted washing of the coat). In such a situation, rodents spontaneously prefer the periphery of the apparatus to activity in the central parts of the open field. Indeed, mice and rats walk close to the walls, a behavior called thigmotaxis. Increase of time spent in the central part as well as of the ratio central/total locomotion or decrease of the latency to enter the central part are indications of anxiolysis. Some authors use a procedure in which the animals are allowed free access to the open field, from a familiar cage (see for example Kopp et al., 1997). In this case, the number of risk assessment postures directed to the

* Corresponding author. Tel.: +33-2-47-36-69-94; fax: +33-2-47-36-72-85.
E-mail address: belzung@univ-tours.fr (C. Belzung).

0014-2999/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0014-2999(03)01272-X

Table 1
Effects of benzodiazepines and other $GABA_A$ pentamer ligands on animals subjected to the open field test

Drug	Mechanisms	Animals	Doses	Routes	Effects	Comments	Reference
Abecarnil	Benzodiazepine receptor α1 selective agonist	Wistar rats	0.01–0.3 mg/kg	i.p., 5 ml/kg	+		Rex et al., 1996
Abecarnil	Benzodiazepine receptor α1 selective agonist	Wistar rats	0.01–0.3 mg/kg	i.p.	0		Nazar et al., 1997
Adinazolam	Triazolobenzodiazepine, Benzodiazepine receptor agonist (its metabolite NDMAD potent as benzodiazepine receptor agonist)	Sprague–Dawley rats	1.5–5 mg/kg	route? twice daily, for 12 days	0	–bilaterally bulbectomized rats –chronic treatment	O'Connor et al., 1985
Adinazolam	Triazolobenzodiazepine, benzodiazepine receptor agonist (its metabolite NDMAD potent as benzodiazepine receptor agonist)	Sprague–Dawley rats	1.5–5 mg/kg	route? twice daily, for 12 days	0	–sham rats –chronic treatment	O'Connor et al., 1985
Adinazolam	Triazolobenzodiazepine, benzodiazepine receptor agonist (in fact, its metabolite NDMAD potent as benzodiazepine receptor agonist)	Sprague–Dawley rats	1st hour: 10 mg/kg 2nd hour: 2 mg/kg	i.p.	+		Broderick et al., 1998
Allopregnanolone	Neurosteroid binding to $GABA_A$ receptor pentamer	Sprague–Dawley rats	500 ng	bilaterally infused in the midbrain central gray	0	ovariectomized estradiol benzoate-treated rats	McCarthy et al., 1995
Allopregnanolone	Neurosteroid binding to $GABA_A$ receptor pentamer	Wistar rats	5 and 10 μg/4μl	i.c.v.	–		Czlonkowska et al., 1999
Alpidem	Selective benzodiazepine receptor α1 receptor partial agonist	Wistar rats	0.1–1–10 mg/kg	i.p.	0		Nazar et al., 1997
Alprazolam	Triazolobenzodiazepine, benzodiazepine receptor agonist	Sprague–Dawley rats	2.5–5 mg/kg	route? twice daily, for 12 days	0	–bilaterally bulbectomized rats –chronic treatment	O'Connor et al., 1985
Alprazolam	Triazolobenzodiazepine, benzodiazepine receptor agonist	Sprague–Dawley rats	2.5–5 mg/kg	route? twice daily, for 12 days	0	–sham rats –chronic treatment	O'Connor et al., 1985
Alprazolam	Triazolobenzodiazepine, benzodiazepine receptor agonist	CD1 mice	0.02 mg/kg	i.p.	+		Lopez et al., 1988
Alprazolam	Triazolobenzodiazepine, benzodiazepine receptor agonist	CD1 mice	0.05 mg/kg	i.p.	–		Lopez et al., 1988

Alprazolam	Triazolobenzodiazepine, benzodiazepine receptor agonist	mice	2 mg/kg/day	osmotic pump; 1–14 days	0	–decrease activity after 1 and 2 days –day 4 to 14: tolerance	Miller et al., 1989b
Alprazolam	Triazolobenzodiazepine, benzodiazepine receptor agonist	CD-1 mice	0.2 mg/kg/day	i.p., for 14 days	0	chronic treatment	Lopez et al., 1992
Barbitol	Barbiturate agonist	Wistar rats	40–60–80 mg/kg	route?	–	decrease frequency of grooming	Barros et al., 1994
β-CCB (*n*-butyl β carboline-3-carboxylate)	Benzodiazepine receptor inverse agonist	A_2G mice	1 to 30 mg/kg	i.p.	–	Dose-dependent	Novas et al., 1988
β-CCB (*n*-butyl β carboline-3-carboxylate)	Benzodiazepine receptor inverse agonist	A_2G mice	3 to 30 mg/kg	i.p.	0	decreased nb of rearings	Novas et al., 1988
β-CCE (ethyl β-carboline-3-carboxylate)	Benzodiazepine receptor partial inverse agonist	Long–Evans rats	10 mg/kg	s.c.	–	Sham-lesioned rats	Podhorna and Franklin, 2000
β-CCM (β-carboline-3-carboxylic acid-*N*-methylamide)	Benzodiazepine receptor inverse agonist	Chickens (*Gallus gallus*)	2.5 mg/kg	i.p.	–		Moriarty, 1995
β-CCM	Benzodiazepine receptor inverse agonist	Wistar rats	0.1–0.5–5 mg/kg	i.p.	–		Nazar et al., 1997
Bicuculline	$GABA_A$ receptor antagonist	Wistar rats	0.25 mg/kg	i.p.	0		Car et al., 1996
Bretazenil (Ro 16-6028)	Benzodiazepine receptor partial agonist	Hooded rats	1–10 mg/kg	i.p.	+	increased rearings and decreased groomings at high dose	Yerbury and Cooper, 1987
Bretazenil	Benzodiazepine receptor partial agonist	mice	0.25, 1 and 4 mg/kg/day	implanted s.c. osmotic pump	0	–dose-dependent –decrease rearings	Miller et al., 1990
Bretazenil	Benzodiazepine receptor partial agonist	Wistar rats	0.1–1–10 mg/kg	i.p.	0		Nazar et al., 1997
Bretazenil	Benzodiazepine receptor partial agonist	Wistar rats	10 μg/site	in the dentate gyrus of the dorsal hippocampus	0	inhibit motor activity	Nazar et al., 1999a,b
Bretazenil	Benzodiazepine receptor partial agonist	Sprague–Dawley rats	200 and 300 μg/kg	i.p.	0	decrease activity (square crossing)	Tashima et al., 2001
Brotizolam	Thienotriazolobenzodiazepine, benzodiazepine receptor agonist	Wistar rats	0.5 mg/kg	p.o.	+		Ueki et al., 1984

(continued on next page)

Table 1 (*continued*)

Drug	Mechanisms	Animals	Doses	Routes	Effects	Comments	Reference
Brotizolam	Thienotriazolobenzodiazepine, benzodiazepine receptor agonist	Wistar rats	1–2 mg/kg	p.o.	0		Ueki et al., 1984
Brotizolam	Thienotriazolobenzodiazepine, benzodiazepine receptor agonist	Wistar rats	5 mg/kg	p.o.	−		Ueki et al., 1984
Chlor-desmethyldiazepam	Benzodiazepine receptor full agonist	Swiss-NOS mice	0.08–1.25–5 mg/kg	i.p.	0.08: 0 1.25: + 5: −	at 5 mg/kg: sedative effect	De Angelis et al., 1982
Chlordiazepoxide	Benzodiazepine, benzodiazepine receptor full agonist	Sprague–Dawley rats	20 and 30 mg/kg	s.c., in 1 ml/kg	+	single food pellet in the center of a new open field environment	Britton and Britton, 1981
Chlordiazepoxide	Benzodiazepine, benzodiazepine receptor full agonist	NIH albino mice	5, 10, 30 and 50 mg/kg	i.p., in 5 ml/kg	+		Crawley, 1981
Chlordiazepoxide	Benzodiazepine, benzodiazepine receptor full agonist	Inbred rats F344	1 mg/kg	i.p., on days 1–21 of pregnancy (perinatally)	0	−activity minimally affected −chronic treatment	Adams, 1982
Chlordiazepoxide	Benzodiazepine, benzodiazepine receptor full agonist	rats	3 mg/kg	i.p.	+		Sanger and Zivkovic, 1988
Chlordiazepoxide	Benzodiazepine, benzodiazepine receptor full agonist	rats	30 mg/kg	i.p.	0	decreased locomotion	Sanger and Zivkovic, 1988
Chlordiazepoxide	Benzodiazepine, benzodiazepine receptor full agonist	Wistar rats	5 mg/kg	i.p.	0	−nonhabituated rats −increased locomotion	Gentsch et al., 1989
Chlordiazepoxide	Benzodiazepine, benzodiazepine receptor full agonist	Wistar rats	5 mg/kg	i.p.	0	habituated rats	Gentsch et al., 1989
Chlordiazepoxide	Benzodiazepine, benzodiazepine receptor full agonist	Wistar rats	100 μg/kg and 0.1 mg/kg	i.p.	+		Bruhwyler, 1990
Chlordiazepoxide	Benzodiazepine, benzodiazepine receptor full agonist	Sprague–Dawley rats	3.75–5–7.5–10 mg/kg	i.p.	+		Horvath et al., 1992
Chlordiazepoxide	Benzodiazepine, benzodiazepine receptor full agonist	Sprague–Dawley rats	2.5,5,10 mg/kg	i.p., 1 ml/kg during 5 days	+	−dose sensitivity −chronic treatment	Angrim et al., 1998

Chlordiazepoxide	Benzodiazepine, benzodiazepine receptor full agonist	CF1 mice	5 and 10 mg/kg	i.p.	?	–switch from "high explore" to "high walk" –reduced stretched posture and increased wall-following	Cholens et al., 2001
Chlordiazepoxide	Benzodiazepine, benzodiazepine receptor full agonist	Wistar rats	1,5,10 mg/kg	i.p.	0	rats exposed to a single session of foot shocks or exposed to the grid cage without shock	Bruinzeel et al., 2001
CL 218,872	Triazolopyridazine, benzodiazepine receptor partial agonist	Long–Evans rats	10–20 mg/kg	i.p. in 2 ml/kg	+	1 and 5 mg/kg doses have no effect	McNamara and Skelton, 1992
Clonazepam	Triazolobenzodiazepine, benzodiazepine receptor agonist	NIH albino mice	0.1, 0.5 and 5 mg/kg	i.p., in 5 ml/kg	+		Crawley, 1981
Clonazepam	Triazolobenzodiazepine, benzodiazepine receptor agonist	CD1 mice	0.02–0.05 mg/kg	i.p.	–	dose dependent	Lopez et al., 1988
Clonazepam	Triazolobenzodiazepine, benzodiazepine receptor agonist	CD-1 mice	1.5 mg/kg/day	for 1–14 days	days 1, 2 and 4: – days 7 and 14: 0	chronic treatment	Galpern et al., 1991
Clonazepam	Triazolobenzodiazepine, benzodiazepine receptor agonist	Wistar rats	0.1–0.2–0.4–0.8 mg/kg	route?	+	decrease frequency of grooming	Barros et al., 1994
Crotoxin (*Crotalus durissus terrificus* venom)	Benzodiazepine receptor inverse agonist	Wistar rats	100, 250 and 500 μg/kg	i.p.	–		Moreira et al., 1996
Crotoxin	Benzodiazepine receptor inverse agonist	Wistar rats	100, 250 and 500 μg/kg	i.p.	–		Moreira et al., 2000
Desmethyldiazepam	Benzodiazepine receptor agonist, metabolite of diazepam	Swiss-NOS mice	0.16–1.25–5 mg/kg	i.p.	0.16: 0 1.15: + 5: –	at 5 mg/kg: sedative effect	De Angelis et al., 1982
DHEA (dehydroepiandrosterone)	Neurosteroid binding to $GABA_A$ receptor pentamer	Long–Evans rats	0, 3, or 7.5 mg/kg	s.c.	0	decreased activity	Frye and Lacey, 1999
5-androstan-3β-ol-17-one sulfate (dihydroepiandrosterone sulfate = DHEAS)	Neurosteroid binding to $GABA_A$ receptor pentamer	Long–Evans rats	3.2 and 6.4 mg/kg	s.c. and i.c.v.	0	ovariectomized rats	Frye and Sturgis, 1995
DHEAS	Neurosteroid binding to $GABA_A$ receptor pentamer	Wistar rats	5 and 20 mg/kg	s.c.	0		Reddy et al., 1998

(continued on next page)

Table 1 (*continued*)

Drug	Mechanisms	Animals	Doses	Routes	Effects	Comments	Reference
DHEAS	Neurosteroid binding to $GABA_A$ receptor pentamer	Long–Evans rats	0, 3, or 7.5 mg/kg	s.c.	0	decreased activity	Frye and Lacey, 1999
Diazepam	Benzodiazepine, benzodiazepine receptor full agonist	Wistar rats, male	5 and 10 mg/kg	s.c., from days 5 to 45 of life; 0.03–0.05 ml from days 5 to 25 and 0.06–0.1 ml from days 26 to 45	0	–chronic treatment –increased ambulation –decreased defecation	Fonseca et al., 1976
Diazepam	Benzodiazepine, benzodiazepine receptor full agonist	Wistar rats, female	5 and 10 mg/kg	s.c., from days 5 to 45 of life; 0.03–0.05 ml from days 5 to 25 and 0.06–0.1 ml from days 26 to 45	–	–chronic treatment –decreased ambulation –increased defecation	Fonseca et al., 1976
Diazepam	Benzodiazepine, benzodiazepine receptor full agonist	Mongolian gerbils (*Meriones unguiculatus*)	8 mg/kg	i.p., 0.34 ml per gerbil	–		Jarbe and Johansson, 1977
Diazepam	Benzodiazepine, benzodiazepine receptor full agonist	NIH albino mice	0.5, 2, 5, 10 and 25 mg/kg	i.p., in 5 ml/kg	+	at 25 mg/kg: sedation	Crawley, 1981
Diazepam	Benzodiazepine, benzodiazepine receptor full agonist	Sprague–Dawley albino rats	1.5 mg/kg	s.c., in 1 ml/kg	+	single food pellet in the center of a new open field environment	Britton and Britton, 1981
Diazepam	Benzodiazepine, benzodiazepine receptor full agonist	Wistar rats	2.5, 5, 10 mg/kg/day	s.c., for 16 days of pregnancy	–longer latencies –decreased rearings	chronic treatment	Gai and Grimm, 1982
Diazepam	Benzodiazepine, benzodiazepine receptor full agonist	inbred strain rats	2.5 mg/kg	p.o.	+		Matsubara and Matsushita, 1982
Diazepam	Benzodiazepine, benzodiazepine receptor full agonist	inbred strain rats	20 mg/kg	p.o.	0	reduced activity	Matsubara and Matsushita, 1982
Diazepam	Benzodiazepine, benzodiazepine receptor full agonist	inbred strain rats	2.5, 5, 10 and 20 mg/kg	p.o., repeated for 2, 4, 7 and 14 days	+		Matsubara and Matsushita, 1982
Diazepam	Benzodiazepine, benzodiazepine receptor full agonist	Wistar rats	0.025, 0.05, 0.1 g/kg	s.c.	+		Hard et al., 1985
Diazepam	Benzodiazepine, benzodiazepine receptor full agonist	Sprague–Dawley rats	2.5 mg/kg	route? twice daily, for 12 days	0	–bilaterally bulbectomized rats –chronic treatment	O'Connor et al., 1985

Diazepam	Benzodiazepine, benzodiazepine receptor full agonist	inbred strains rats	2.5 mg/kg	route? twice daily, for 12 days	0	–sham rats –chronic treatment	O'Connor et al., 1985
Diazepam	Benzodiazepine, benzodiazepine receptor full agonist	inbred strains rats	1–5 mg/kg	p.o.	+		Defini-Stula and Hunn, 1988
Diazepam	Benzodiazepine, benzodiazepine receptor full agonist	A_2G mice	0.3 mg/kg	i.p.	+		Novas et al., 1988
Diazepam	Benzodiazepine, benzodiazepine receptor full agonist	rats	2.5 mg/kg/day	prenatally or postnatally	+		Guillamon et al., 1990
Diazepam	Benzodiazepine, benzodiazepine receptor full agonist	inbred strains rats	100 μg/kg	i.p.	0	inhibition of ambulation	Bruhwyler, 1990
Diazepam	Benzodiazepine, benzodiazepine receptor full agonist	Sprague–Dawley rats	1 mg/kg	i.p.	0	non-stressed rats	Pohorecky and Roberts, 1991
Diazepam	Benzodiazepine, benzodiazepine receptor full agonist	Sprague–Dawley rats	5 mg/kg	i.p.	–	non-stressed rats	Pohorecky and Roberts, 1991
Diazepam	Benzodiazepine, benzodiazepine receptor full agonist	Sprague–Dawley rats	1 mg/kg	i.p.	+	stressed rats	Pohorecky and Roberts, 1991
Diazepam	Benzodiazepine, benzodiazepine receptor full agonist	Sprague–Dawley rats	5 mg/kg	i.p.	–	stressed rats	Pohorecky and Roberts, 1991
Diazepam	Benzodiazepine, benzodiazepine receptor full agonist	Long–Evans rats	3 mg/kg	i.p. in 2 ml/kg	+		McNamara and Skelton, 1992
Diazepam	Benzodiazepine, benzodiazepine receptor full agonist	Wistar rats	1 and 2 mg/kg	i.p., 1 ml/kg	0	at 2 mg/kg, walking and rearing were decreased	Hughes, 1993
Diazepam	Benzodiazepine, benzodiazepine receptor full agonist	Wistar rats	5 mg/kg/day	i.p., for 14 and 28 days	+	chronic treatment	Sherif and Oreland, 1994
Diazepam	Benzodiazepine, benzodiazepine receptor full agonist	Sprague–Dawley rats	100 ng	bilaterally infused in the midbrain central gray	+	ovariectomized estradiol benzoate-treated rats	McCarthy et al., 1995
Diazepam	Benzodiazepine, benzodiazepine receptor full agonist	rats	10 mg/kg	i.p., given from days 13 to 20 of gestation	–	chronic treatment	Singh et al., 1996
Diazepam	Benzodiazepine, benzodiazepine receptor full agonist	Wistar rats	2.5–5 mg/kg	i.p., 5 ml/kg	+		Rex et al., 1996

(continued on next page)

Table 1 (*continued*)

Drug	Mechanisms	Animals	Doses	Routes	Effects	Comments	Reference
Diazepam	Benzodiazepine, benzodiazepine receptor full agonist	Wistar rats	0.05–1 mg/kg	i.p.	+		Nazar et al., 1997
Diazepam	Benzodiazepine, benzodiazepine receptor full agonist	chicks (Cobb Harding)	0.05, 0.1 or 0.2 mg/kg	i.p.	0		Marin et al., 1997
Diazepam	Benzodiazepine, benzodiazepine receptor full agonist	chicks (Cobb Harding)	0.5 or 1 mg/kg	i.p.	−	sedation	Marin et al., 1997
Diazepam	Benzodiazepine, benzodiazepine receptor full agonist	Sprague–Dawley rats	1st hour: 1 mg/kg 2nd hour: 3 mg/kg	i.p.	0	sedation	Broderick et al., 1998
Diazepam	Benzodiazepine, benzodiazepine receptor full agonist	Charles Foster albino rats	0.25 mg/kg	i.p.	−	streptozotocin-induced diabetic rats	Ramanathan et al., 1998
Diazepam	Benzodiazepine, benzodiazepine receptor full agonist	Charles Foster albino rats	1 mg/kg	i.p.	+	streptozotocin-induced diabetic rats	Ramanathan et al., 1998
Diazepam	Benzodiazepine, benzodiazepine receptor full agonist	Charles Foster albino rats	0.25–1 mg/kg	i.p.	+	non-diabetic rats	Ramanathan et al., 1998
Diazepam	Benzodiazepine, benzodiazepine receptor full agonist	Sprague–Dawley–Hsd rats	2 and 5 mg/kg	i.p., in 2 ml	+		Schmitt and Hiemke, 1998
Diazepam	Benzodiazepine, benzodiazepine receptor full agonist	PVG/OlaHsd	2 and 5 mg/kg	i.p., in 2 ml	+		Schmitt and Hiemke, 1998
Diazepam	Benzodiazepine, benzodiazepine receptor full agonist	Wistar rats	0.5, 1.25, 2.5 and 5 mg/kg	i.p.	+ (except at 5 mg/kg)	bilateral electrical stimulation in the medial prefrontal cortex	Nakamura-Palacios et al., 1999
Diazepam	Benzodiazepine, benzodiazepine receptor full agonist	PVG/OlaHsd rats	1.5 mg/kg	i.p.	+		Schmitt et al., 2000
Diazepam	Benzodiazepine, benzodiazepine receptor full agonist	Wistar rats	0.05, 0.2, 1.5 mg/kg	i.p.	0	decrease motor activity	Siemiatkowski et al., 2000
Diazepam	Benzodiazepine, benzodiazepine receptor full agonist	pig (Landrace × Yorkshire)	0.8 mg/kg	im	0		Andersen et al., 2000
Diazepam	Benzodiazepine, benzodiazepine receptor full agonist	mice	2 mg/kg/day	during 14 days	+	–no stimulant effect on locomotion –chronic treatment	Boerngen-Lacerda and Souza-Formigoni, 2000
Diazepam	Benzodiazepine, benzodiazepine receptor full agonist	CF1 mice	1.5 mg/kg	i.p.	+	alterations in sit and groom	Choleris et al., 2001

Diazepam	Benzodiazepine, benzodiazepine receptor full agonist	Sprague–Dawley rats	400 μg/kg	i.p.	0	decrease activity (square crossing)	Tashma et al., 2001
Diazepam	Benzodiazepine, benzodiazepine receptor full agonist	Wistar rats	0.03–10 mg/kg	i.p.	0	aged rats (24 months old)	Wikinski et al., 2001
Diazepam	Benzodiazepine, benzodiazepine receptor full agonist	Fischer 344 rats	0.5–4 mg/kg	i.p.	+		Bert et al., 2001
Diazepam	Benzodiazepine, benzodiazepine receptor full agonist	Harlan–Wistar rats	0.5–4 mg/kg	i.p.	+		Bert et al., 2001
Diazepam	Benzodiazepine, benzodiazepine receptor full agonist	Wistar rats	2 mg/kg	i.p.		increased motor activity	Beaufour et al., 2001
Diazepam	Benzodiazepine, benzodiazepine receptor full agonist	Wistar rats, male	1.5 mg/kg	s.c., over gestation days 14–20	+	–non-handled (NH) –chronic treatment	Cannizzaro et al., 2001
Diazepam	Benzodiazepine, benzodiazepine receptor full agonist	Wistar rats, female	1.5 mg/kg	s.c., over gestation days 14–20	–	–non-handled (NH) –chronic treatment	Cannizzaro et al., 2001
Diazepam	Benzodiazepine, benzodiazepine receptor full agonist	Wistar rats, male and female	1.5 mg/kg	s.c., over gestation days 14–20	Slightly influenced	–short-lasting handled (SLH) –chronic treatment	Cannizzaro et al., 2001
Diazepam	Benzodiazepine, benzodiazepine receptor full agonist	Wistar rats, male	1.5 mg/kg	s.c., over gestation days 14–20	+	–long-lasting handled (LLH) –chronic treatment	Cannizzaro et al., 2001
Diazepam	Benzodiazepine, benzodiazepine receptor full agonist	Wistar rats, female	1.5 mg/kg	s.c., over gestation days 14–20	+	–long-lasting handled (LLH) –chronic treatment	Cannizzaro et al., 2001
Diazepam	Benzodiazepine, benzodiazepine receptor full agonist	Broiler chick (Cobb Harding hybrid)	0.05 mg/kg	i.p.	0	low latency to peck pebbles	Salvatierra and Arce, 2001
Diazepam	Benzodiazepine, benzodiazepine receptor full agonist	Broiler chick (Cobb Harding hybrid)	0.05 mg/kg	i.p.	+	moderate latency to peck pebbles	Salvatierra and Arce, 2001
Diazepam	Benzodiazepine, benzodiazepine receptor full agonist	Broiler chick (Cobb Harding hybrid)	0.05 mg/kg	i.p.	+	high latency to peck pebbles	Salvatierra and Arce, 2001
Doramectin	$GABA_A$ receptor agonist	Wistar rats	100, 300 and 1000 μg/kg	s.c.	0	–few alterations in locomotion frequency –reduction of grooming	De Souza Spinosa et al., 2000
Estazolam	Triazolobenzodiazepine, benzodiazepine receptor agonist	Wistar rats	0.5–1 mg/kg	p.o.	+		Ueki et al., 1984
Estazolam	Triazolobenzodiazepine, benzodiazepine receptor agonist	Wistar rats	10 mg/kg	p.o.	0		Ueki et al., 1984

(continued on next page)

Table 1 (*continued*)

Drug	Mechanisms	Animals	Doses	Routes	Effects	Comments	Reference
Ethanolamine *O*-sulfate (EOS)	GABA-Transaminase inhibitor	inbred rats	200 and 400 μg	intracisternal	0	decreased activity	Nobrega and Coscina, 1983
EOS	GABA-Transaminase inhibitor	inbred strains rats	100 followed by 0 followed by 200 μg in 20 μl deionized water	intracisternal injection in the lateral hypothalamus, 1 week separating each injection	0	decreased activity	Coscina and Nobrega, 1989
EOS	GABA-Transaminase inhibitor	Wistar rats	200 mg/kg/day	from postnatal days 3 to 21	0	–chronic treatment –reduced activity	Taira et al., 1992
FG-7142 (*N*-methyl-β-carboline-3-carboxamide)	Benzodiazepine receptor inverse agonist	inbred strains rats	1, 5, 10, 30 mg/kg	i.p.	0	–increased ambulation –increased rearings	Bruhwyler et al., 1991
FG-7142	Benzodiazepine receptor inverse agonist	mice	20 mg/kg/day	implanted s.c. osmotic pump for 1 to 14 days	–days 1 and 2: 0 –days 4 and 7: + –day 14: –	chronic treatment	Pritchard et al., 1991
FG 7142	Benzodiazepine receptor inverse agonist	Sprague–Dawley rats, male	5,10 and 20 mg/kg	i.p., in 2 ml/kg	–		Meng and Drugan, 1993
FG 7142	Benzodiazepine receptor inverse agonist	Sprague–Dawley rats, female	40 mg/kg	i.p., in 2 ml/kg	–		Meng and Drugan, 1993
FG 7142	Benzodiazepine receptor inverse agonist	Chicks (Cobb Harding)	0.1 and 1 mg/kg	i.p., 0.2 ml/100 g	–		Marin et al., 1997
Flumazenil (Ro 15-1788)	Benzodiazepine receptor antagonist	mice	1 and 5 mg/kg/day	implanted s.c. osmotic pump for 14 days	days 1, 2 and 4: 0	chronic treatment	Miller et al., 1989a
Flumazenil	Benzodiazepine receptor antagonist	mice	2 mg/kg/day	implanted s.c. osmotic pump for 14 days	days 7 and 14: +	chronic treatment	Miller et al., 1989a
Flumazenil	Benzodiazepine receptor antagonist	RLA/Verh rats	3.5 and 6.3 mg/kg/day	from day 15 to the 14th day after birth	0	chronic treatment	Ferre et al., 1996
Flumazenil	Benzodiazepine receptor antagonist	Wistar rats	0.1–1–10 mg/kg	i.p.	0		Nazar et al., 1997
Flurazepam	Benzodiazepine, benzodiazepine receptor full agonist	NIH albino mice	1, 5, 10 and 20 mg/kg	i.p., in 5 ml/kg	+		Crawley, 1981
Flurazepam	Benzodiazepine, benzodiazepine receptor full agonist	Sprague–Dawley albino rats	5, 10 and 20 mg/kg	s.c., in 1 ml/kg	0		Britton and Britton, 1981

Girisopam: GYKI 51,189(EGIS 5810): (1-(3-chlorophenyl)-4-methyl-7, 8-dimethoxy-5H-2,3-benzodiazepine)	2,3-Benzodiazepine, benzodiazepine receptor agonist	Sprague–Dawley rats	37.5–50 mg/kg	i.p.	+		Horvath et al., 1992
GYKI 52,322 (EGIS 6775): (1-(4-aminophenyl)-4-methyl-7,8-dimethoxy-5H-2,3-benzodiazepine)	2,3-Benzodiazepine, benzodiazepine receptor agonist	Sprague–Dawley rats	2.5–5–7.5–10 mg/kg	i.p.	+		Horvath et al., 1992
Lorazepam	Benzodiazepine, benzodiazepine receptor full agonist	Lister rats	0.25 or 1.25 mg/kg	s.c., between postnatal days 7 and 21	0	chronic treatment	File and Tucker, 1983
Lorazepam	Benzodiazepine, benzodiazepine receptor full agonist	Mice, 3 weeks old	2 mg/kg/day	from days 13 to 20 of gestation	0	–prenatal injection –chronic treatment –increased activity	Chesley et al., 1991
Lorazepam	Benzodiazepine, benzodiazepine receptor full agonist	Mice, 6 weeks old	2 mg/kg/day	from days 13 to 20 of gestation	0	–prenatal injection –chronic treatment	Chesley et al., 1991
Lorazepam	Benzodiazepine, benzodiazepine receptor full agonist	inbred ICR mice	0.2–2 mg/kg	i.p.	0	Dose-dependent decreased activity	Fahey et al., 1999
Lorazepam	Benzodiazepine, benzodiazepine receptor full agonist	Mice (CD 1(ICR)BR)	2 mg/kg	pump implanted subcutaneously	0	–total distance travelled decrease –nb of rearings decrease –total stereotypies decrease	Fahey et al., 2001
Lormetazepam	Benzodiazepine, benzodiazepine receptor full agonist	inbred ICR mice	0.2–2 mg/kg	i.p.	0	Dose-dependent decreased activity	Ueki et al., 1985
Midazolam	Benzodiazepine, benzodiazepine receptor full agonist	Hooded rats	1–10 mg/kg	i.p.	0	dose dependently reduced general activity	Yerbury and Cooper, 1987
Midazolam	Benzodiazepine, benzodiazepine receptor full agonist	Wistar rats	0.01 and 0.1μg	in the hippocampus	+		Stefanski et al., 1993
Midazolam	Benzodiazepine, benzodiazepine receptor full agonist	Wistar rats	0.01 and 0.1μg	in the nucleus accumbens septi	0		Stefanski et al., 1993
Midazolam	Benzodiazepine, benzodiazepine receptor full agonist	Wistar rats	0.01–0.1–0.5–1 mg/kg	i.p.	+		Nazar et al., 1997
Midazolam	Benzodiazepine, benzodiazepine receptor full agonist	Wistar rats	0.5 μg/4μl	i.c.v.	+		Członkowska et al., 1999

(continued on next page)

Table 1 (*continued*)

Drug	Mechanisms	Animals	Doses	Routes	Effects	Comments	Reference
Midazolam	Benzodiazepine, benzodiazepine receptor full agonist	Wistar rats	0.1 μg/site	in the dentate gyrus of the dorsal hippocampus	+		Nazar et al., 1999a,b
Midazolam	Benzodiazepine, benzodiazepine receptor full agonist	Wistar rats	10 μg/site	in the dentate gyrus of the dorsal hippocampus	0	inhibit motor activity	Nazar et al., 1999a,b
Muscimol	$GABA_A$ receptor agonist	Wistar rats	0.2 mg/kg	between the 1st and the 21st postnatal days	0	decreased activity	Taira et al., 1993
Muscimol	$GABA_A$ receptor agonist	Wistar rats	1 m/kg	p.o.	0	rats prenatally exposed to delta9-tetrahydrocannabinol or oil	Garcia-Gil et al., 1999
Muscimol	$GABA_A$ receptor agonist	Wistar rats	0.5 and 1 μg per side	bilateral infusion into the ventral hippocampus	0	–dose-dependent –decreased activity	Bast et al., 2001b
Nitrazepam	Benzodiazepine, benzodiazepine receptor full agonist	Wistar rats	1 mg/kg	p.o.	+	slight effect	Ueki et al., 1984
Nitrazepam	Benzodiazepine, benzodiazepine receptor full agonist	Wistar rats	2 mg/kg	p.o.	+	decrease ambulation 2 and 4 h after administration	Ueki et al., 1984
Nitrazepam	Benzodiazepine, benzodiazepine receptor full agonist	Wistar rats	20 mg/kg	p.o.	+	increase ambulation 2 h after administration	Ueki et al., 1984
Oxazepam	Benzodiazepine, benzodiazepine receptor full agonist	mice	5, 15 and 50 mg/kg twice daily	p.o., from 12 to 16 of pregnancy	0	–chronic treatment –at 60 days: reduction of locomotion	Alleva et al., 1985
Oxazepam	Benzodiazepine, benzodiazepine receptor full agonist	mice	5, 15 and 50 mg/kg twice daily	p.o., from 12 to 16 of pregnancy	0	–chronic treatment –at 14–16 days: reduced activity	Alleva et al., 1985
Oxazepam	Benzodiazepine, benzodiazepine receptor full agonist	CD-1 mice	15 mg/kg	p.o., twice a day, on days 12–16 of fetal life	0	chronic treatment	Laviola et al., 1992
Oxazepam	Benzodiazepine, benzodiazepine receptor full agonist	CD-1 mice	15 mg/kg	p.o., twice a day, on days 12–16 of fetal life	0	–chronic treatment –increase frequency of grooming, rearing, sniffing –reduced walking	Fiore et al., 1995
Pentabarbitol	Barbiturate agonist	Mongolian gerbils (*Meriones unguiculatus*)	15 and 20 mg/kg	i.p., 0.34 ml per gerbil	+		Jarbe and Johansson, 1977

Pentabarbitol	Barbiturate agonist	Sprague–Dawley albino rats	5 and 10 mg/kg	s.c., in 1 ml/kg	+	single food pellet in the center of a new open field environment	Britton and Britton, 1981
Pentabarbitol	Barbiturate agonist	NIH albino mice	40 mg/kg	i.p., in 5 ml/kg	+		Crawley, 1981
Phenazepam	Benzodiazepine, benzodiazepine receptor agonist	C57BL/6 mice	0.05, 0.075, 0.1 mg/kg	i.p.	–	Dose-dependent suppression of locomotor activity	Seredenin et al., 1990
Phenazepam	Benzodiazepine, benzodiazepine receptor agonist	BALB/c mice	0.05 mg/kg	i.p.	+		Seredenin et al., 1990
Phenazepam	Benzodiazepine, benzodiazepine receptor agonist	BALB/c mice	0.075–0.1 mg/kg	i.p.	0		Seredenin et al., 1990
Phenazepam	Benzodiazepine, benzodiazepine receptor agonist	F1 (C57BL/6 × BALB/c) mice	0.05 mg/kg	i.p.	0	Dose-dependent suppression of locomotor activity	Seredenin et al., 1990
Phenazepam	Benzodiazepine, benzodiazepine receptor agonist	F1 (C57BL/6 × BALB/c) mice	0.075, 0.1 mg/kg	i.p.	–	Dose-dependent suppression of locomotor activity	Seredenin et al., 1990
Phenobarbitol	Barbiturate agonist	Wistar rats, male	10 and 20 mg/kg	s.c., from days 5 to 45 of life; 0.03–0.05 ml from days 5 to 25 and 0.06–0.1 ml from days 26 to 45	+ (decreased defecation)	–chronic treatment –increased ambulation	Fonseca et al., 1976
Phenobarbitol	Barbiturate agonist	Wistar rats, female	10 and 20 mg/kg	s.c., from days 5 to 45 of life; 0.03–0.05 ml from days 5 to 25 and 0.06–0.1 ml from days 26 to 45	+ (increased defecation)	–chronic treatment –decreased ambulation	Fonseca et al., 1976
Picrotoxin	Picrotoxin and *t*-butylbicyclophosphorothionate binding site on $GABA_A$ receptor pentamert	inbred strain rats	25 and 50 ng in 0.25 μl	in the midbrain periaqueductal gray matter	0	–increased backward locomotion –decreased grooming	Depaulis and Vergnes, 1986
Picrotoxin	Picrotoxin and *t*-butylbicyclophosphorothionate binding site on $GABA_A$ receptor pentamert	Sprague–Dawley rats	0.5–1 mg/kg	i.p. in 2 ml/kg	0		Fernandez-Teruel et al., 1990
Picrotoxin	Picrotoxin and *t*-butylbicyclophosphorothionate binding site on $GABA_A$ receptor pentamert	Wistar rats	0.75 mg/kg	s.c., on day 18 of pregnancy and daily during the first 5 days of lactation	0	–chronic treatment –hyperactivity	Silva et al., 1995

(continued on next page)

Table 1 (*continued*)

Drug	Mechanisms	Animals	Doses	Routes	Effects	Comments	Reference
Picrotoxin	Picrotoxin and *t*-butylbicyclophosphorothionate binding site on $GABA_A$ receptor pentamer	Wistar rats	0.1 μg/site	in the dentate gyrus of the dorsal hippocampus	+		Nazar et al., 1999a,b
Picrotoxin	Picrotoxin and *t*-butylbicyclophosphorothionate binding site on $GABA_A$ receptor pentamer	Wistar rats	150 ng/0.5 μl per side	bilateral infusions into the ventral hippocampus	+		Bast et al., 2001a
Piracetam	GABA derivative compound	Wistar rats	250 and 500 mg/kg	p.o., for 7 and 14 days	+	chronic treatment	Bhattacharya et al., 1993
Piracetam	GABA derivative compound	Wistar rats	250 and 500 mg/kg	p.o.	0		Bhattacharya et al., 1993
3α-hydroxy-5α-pregna-20-one (3α, 5α THP)	Neurosteroid binding to $GABA_A$ receptor pentamer	mice	1 or 2 μg	i.c.v.	0		Khisti et al., 2000
3α, 5α THP	Neurosteroid binding to $GABA_A$ receptor pentamer	mice	0.5, 1, 2 mg/kg	i.p.	0		Khisti et al., 2000
5α-pregnan-3α-ol-20-one (THP)	Neurosteroid binding to $GABA_A$ receptor pentamer	Long–Evans rats	3.2 or 6.4 mg/kg	s.c.	0		Frye and Sturgis, 1995
5α-THDOC (3α-21-dihydroxy-5α-pregnanolone or α-tetrahydrodeoxycorticosterone)	Neurosteroid binding to $GABA_A$ receptor pentamer	Wistar rats	10 and 20 μg/4μl	i.c.v.	−		Członkowska et al., 1999
5β-THDOC (3α-21-dihydroxy-5β-pregnanolone, 5βtetrahydrodeoxycorticosterone)	Neurosteroid binding to $GABA_A$ receptor pentamer	Wistar rats	5 and 10 μg/4μl	i.c.v.	−		Członkowska et al., 1999
5α-pregnan-3α-ol-11,20-dione	Neurosteroid binding to $GABA_A$ receptor pentamer	Long–Evans rats	3.2 or 6.4 mg/kg	s.c.	0		Frye and Sturgis, 1995
4-pregnen-3, 20-dione-17α-hydroxyprogesterone	Neurosteroid binding to $GABA_A$ receptor pentamer	Long–Evans rats	3.2 or 6.4 mg/kg	s.c.	0		Frye and Sturgis, 1995
5-pregnen-3β-ol-20-one sulfate	Neurosteroid binding to $GABA_A$ receptor pentamer	Long–Evans rats	3.2 or 6.4 mg/kg	s.c.	0		Frye and Sturgis, 1995
pregnenolone sulfate (PS)	Neurosteroid binding to $GABA_A$ receptor pentamer	Wistar rats	5 mg/kg	s.c.	0		Reddy et al., 1998
PS	Neurosteroid binding to $GABA_A$ receptor pentamer	Wistar rats	5, 10 and 20 μg/4μl	i.c.v.	+	Dose-dependent	Członkowska et al., 1999

Progesterone	Neurosteroid binding to $GABA_A$ receptor pentamer	inbred strains mice	10 mg/kg	s.c.	0		Reddy et al., 1998
R05-4864	Peripheric benzodiazepine receptor ligand	NIH albino mice	20 mg/kg	i.p., in 5 ml/kg	0		Crawley, 1981
Ro 11-6893	1,4-Benzodiazepine, Ro 11-6896, inactive stereoisomer of Ro 11-6896	Mongolian gerbils (*Meriones unguiculatus*)	10 mg/kg	i.p., 2 injections of 4 ml/kg	0		Hiltunen and Jarbe, 1986
Ro 11-6896	1,4-Benzodiazepine, Benzodiazepine receptor agonist	Mongolian gerbils (*Meriones unguiculatus*)	1 mg/kg	i.p., 2 injections of 4 ml/kg	0		Hiltunen and Jarbe, 1986
Ro 15-4513	Benzodiazepine receptor inverse agonist	Charles River rats	2.5 mg/kg	i.p.	0		June and Lewis, 1989
Ro 15-4513	Benzodiazepine receptor inverse agonist	inbred strains rats	1.25 and 2.5 mg/kg	i.p.	0		June et al., 1989
Ro 17-1812	Benzodiazepine receptor partial agonist	Hooded rats	1–10 mg/kg	i.p.	0	decreased grooming at high dose	Yerbury and Cooper, 1987
Ro 19-8022	Benzodiazepine receptor partial agonist	Wistar rats	0.1–0.5–1–10 mg/kg	i.p.	0		Nazar et al., 1997
RY 008	Benzodiazepine receptor partial inverse agonist	Wistar rats	50 and 500 ng	intrastriatal injection	0		June et al., 1998
SKF 89976-A	GABA uptake inhibitor	PVG/OlaHsd rats	5–10–15–20–25 mg/kg	i.p., injected in 2.5 ml	+		Schmitt and Hiemke, 1999
SKF-89976-A	GABA uptake inhibitor	PVG/OlaHsd rats	15 mg/kg	i.p.	+		Schmitt et al., 2001
SR 95531	$GABA_A$ receptor antagonist	Wistar rats	50 ng	intrastriatal injection	0		June et al., 1998
Testosterone	Neurosteroid binding to $GABA_A$ receptor pentamer	Wistar rats (immature, 6 weeks old)	40 μg/100 g body weight	i.p., once daily for 3 consecutive days	0	–observed at 4 and at 24 h after injection –inhibit horizontal and vertical locomotor activity	Lambadjieva, 1998
Testosterone	Neurosteroid binding to $GABA_A$ receptor pentamer	Wistar rats (immature, 6 weeks old)	40 μg/100 g body weight	i.p., once daily for 3 consecutive days	0	–continual light leads –observed at 4 and 24 h after injection –increased horizontal and vertical locomotor activity	Lambadjieva, 1999
THBC (1,2,3,4-tetrahydro-β-carboline)	Benzodiazepine receptor inverse agonist	inbred strains rats	10 or 50 ng in 3 μl	bilaterally into the hippocampus	0	reduced motor activity	Huttunen and Myers, 1986
THBC (1,2,3,4-tetrahydro-β-carboline)	Benzodiazepine receptor inverse agonist	inbred strains rats	50 ng in 3 μl	bilaterally into the hippocampus	+ (increase time of freezing-immobilisation)		Huttunen and Myers, 1986
THIP (4,5,6,7-tetrahydroxi-azolo-5,4*c*-pyridine-3-ol)	$GABA_A$ receptor agonist	inbred strain rats	20 mg/kg	s.c.	0	depressant effect	Borsini et al., 1988

(continued on next page)

Table 1 (*continued*)

Drug	Mechanisms	Animals	Doses	Routes	Effects	Comments	Reference
THIP	$GABA_A$ receptor agonist	Wistar rats	0.5–0.75–1 mg/kg	route?	0	decrease frequency of grooming	Barros et al., 1994
Tiagabine	GABA uptake inhibitor	rats	18.5 mg/kg	i.p.	+		Schmitt and Hiemke, 1999
Tiagabine	GABA uptake inhibitor	PVG/OlaHsd rats	4.5 mg/kg	i.p.	0		Schmitt. et al., 2000
Tiagabine	GABA uptake inhibitor	PVG/OlaHsd rats	18.5 mg/kg	i.p.	+		Schmitt et al., 2000
Triazolam	Triazolobenzodiazepine, Benzodiazepine receptor agonist	Mice CD1	0.02–0.05 mg/kg	i.p.	–	dose-dependent fashion	Lopez et al., 1988
Vigabatrin	GABA-Transaminase inhibitor	Wistar rats	50 mg/kg	i.p.	+	observed 2,4 and 24 h after injection	Sherif and Oreland, 1994
Vigabatrin	GABA-Transaminase inhibitor	Wistar rats	50 mg/kg/day	i.p., for 14 and 28 days	0	chronic treatment	Sherif and Oreland, 1994
Vigabatrin	GABA-Transaminase inhibitor	Wistar rats	250 mg/kg	i.p.	+	isolated rats	Sherif and Oreland, 1995
Vigabatrin	GABA-Transaminase inhibitor	Wistar rats	250 mg/kg	i.p.	0	socially housed rats	Sherif and Oreland, 1995
Zolpidem	Selective Benzodiazepine receptor α1 receptor partial agonist	Wistar rats	0.3–3 mg/kg	i.p., 2 ml/kg	0	decrease locomotion	Sanger and Zivkovic, 1988
Zolpidem	Selective Benzodiazepine receptor α1 receptor partial agonist	Wistar rats	0.005–0.001–0.1–1 mg/kg	i.p.	0		Nazar et al., 1997
Zolpidem	Selective Benzodiazepine receptor α1 receptor partial agonist	PVG/OlaHsd and Sprague–Dawley–Hsd rats	0.05 mg/kg	i.p.	+		Schmitt and Hiemke, 1998
Zolpidem	Selective Benzodiazepine receptor α1 receptor partial agonist	PVG/OlaHsd and Sprague–Dawley–Hsd rats	3 mg/kg	i.p.	0	decrease activity	Schmitt and Hiemke, 1998
Zolpidem	Selective Benzodiazepine receptor α1 receptor partial agonist	Wistar rats	10 μg/site	in the dentate gyrus of the dorsal hippocampus	–	decrease motor activity	Nazar et al., 1999a,b
Zolpidem	Selective Benzodiazepine receptor α1 receptor partial agonist	Wistar rats	0.1 mg/kg	i.p.	–		Siemiatkowski et al., 2000
Zolpidem	Selective Benzodiazepine receptor α1 receptor partial agonist	Wistar rats	2 mg/kg	i.p.	–		Siemiatkowski et al., 2000
Zolpidem	Selective Benzodiazepine receptor α1 receptor partial agonist	PVG/OlaHsd rats	0.05 mg/kg	i.p., in 2.5 ml	0		Schmitt et al., 2000
Zopiclone	Cyclopyrrolone derivative, $GABA_A$ receptor complex modulator	ddY mice	20 mg/kg	p.o.	0	decrease of activity and rearing at high doses	Ueki, 1987

+, Anxiolytic-like effect; –, anxiogenic-like effect; 0, no anxiolytic or anxiogenic-like effects (in some cases, non-specific effects can be observed but this is specified in the "comment" column); i.p., intraperitoneal; p.o., per os; s.c., subcutaneous; i.c.v., intracerebroventricular.

open field may provide a good measure of the approach response toward novelty, that is, exploration.

The open field has become so popular that its use has been extended to a great number of species, including calves, pigs, lambs, rabbits, pullets, primates, bush babies, honeybees and lobsters. In fact, it has become a convenient procedure to measure not only anxiety-like behaviors, but also sedation or activity. In fact, anxiety behavior in the open field is triggered by two factors: individual testing (the animal is separated from its social group) and agoraphobia (as the arena is very large relative to the animal's breeding or natural environment). It is clear that these two factors may trigger anxiety behavior only in gregarious species and/or in species that show fear of open spaces into which they are forced. This is precisely the case with rodents that live in social groups and in small tunnels. This is of course not the case in species such as lambs or cows that live in large fields. For these reasons, in experiments involving rodents, observers are not measuring the effects of treatments on exploration, as is sometimes claimed, but the effects on the reaction of the subjects to a stressful event. Therefore, anxiolytic treatments do not themselves increase exploration in the open field but they decrease the stress-induced inhibition of exploration behavior.

Behavior of rodents in the open field depends mainly on the tactile sensory factors. Indeed, mice without vibrissae no longer show thigmotaxic behavior, as they lose tactile contact with the walls (unpublished data). They therefore display an increased percent of entries in the central area, which could be interpreted as anxiolytic-like behavior. One must thus emphasize the possibility of misinterpretation of data related to effects of some treatments on the sensory characteristics of the animals. It should also be noted that exploration can be increased by some factors such as food or water deprivation: it is therefore very important to verify that a given treatment does not act on such variables, before concluding about possible effects on anxiety-like behaviors. Finally, open field behavior also depends on lighting conditions and the light–dark cycle, so that it may be relevant to ensure that a treatment does not modify internal clock-related behaviors and to test the treatment under different lighting conditions.

The effects of many different drugs have been investigated in the open field, including compounds with effective or potential anxiolytic effects (benzodiazepines, serotonin ligands, neuropeptides) but also compounds with stimulant (amphetamine, cocaine), sedative (neuroleptic) or prostration-inducing (epileptogenic drugs) activity. An increase in central locomotion or in time spent in the central part of the device without modification of total locomotion and of vertical exploration can be interpreted as an anxiolytic-like effect while the contrary, that is a decrease of these variables, is associated with anxiogenic effects. Increased locomotion can be considered a stimulant effect while decreased vertical activity and locomotion are related to sedation or to post-ictal prostration. It should be said here that the decrease in vertical exploration appears at lower doses than does the decrease in rearing, so that this variable can be considered a more sensitive one. In this paper, we will focus on the effects of pharmacological treatment on anxiety measures in the open field and not on their sedative or stimulant effects. Therefore, this is not a general review on drugs in the open field, but a review of the effects of drugs on anxiety-like variables in the open field. The action of three classes of pharmacological compounds will be reviewed: the effects of compounds acting on the $GABA_A$ pentamer (mainly benzodiazepine receptor ligands but also $GABA_A$ receptor, barbiturate and neurosteroid ligands), the effects of drug acting like 5-hydroxytryptamine (5-HT) (ligands of the different 5-HT receptors as well as selective serotonin reuptake inhibitors, neurotoxins of 5-HT, etc.) and the effects of neuropeptidergic ligands (corticotropin releasing factor: CRF, cholecystokinin: CCK, neurokinin: NK, neuropeptide Y, etc).

2. Effects of compounds acting on the $GABA_A$ pentamer

Classical benzodiazepines are widely used for the clinical treatment of anxiety. They act via the benzodiazepine receptors which are present on the $GABA_A$ pentameric complex. The $GABA_A$ receptors can be allosterically modulated by compounds binding to at least six different sites: the benzodiazepine receptors, a binding site for barbiturates, a site for neurosteroids, a site for the convulsant drugs, picrotoxin and *t*-butylbicyclophosphorothionate, one for flurosemide and one for loreclezole (see Belzung et al., 2002 for more details). In the clinic, benzodiazepine receptor agonists and barbiturate receptor agonists have been shown to display an anxiolytic action while no such effects were seen with other ligands of the pentamer, including $GABA_A$ receptor agonists. The effects of compounds binding on different parts of the $GABA_A$ pentamer in animals subjected to the open field are summarized in Table 1.

Acute administration of benzodiazepine receptor full agonists mostly induces anxiolytic-like effects as they elicit an increase of the percent of entries in the central part of the open field (56% of the studies). However, in some case, these drugs also have no effects (31% of the studies) or even anxiogenic effects (13%). Chronic injection of these compounds mostly does not elicit any effect (66% of the studies). The most used compound is diazepam (52% of the studies investigating the action of a benzodiazepine full agonist). This range of effects (from anxiolytic-like to anxiogenic-like) may be related to the dose used (high doses induce non-specific sedative effects) and also to subtle differences in species or in procedures. For example, moderate doses of benzodiazepines are known to decrease activity in rats and to increase it in mice; this can lead to non-specific modifications in the number of entries in the central part of the apparatus. To avoid this it may be useful to calculate the percent of central entries, rather than the number of entries per se. Some species seem inappropriate for the assessment of anxiolytic effects in the open field: for

Table 2
Effects of ligand acting upon serotonergic neurotransmission on animals subjected to the open field test

Drug	Mechanism	Animals	Doses	Routes	Effects	Comments	Reference
(−)Pindolol	Non-selective antagonist	Sprague–Dawley rats (200–250 g)	10	i.p.	+	Locomotion increased	Lucki et al., 1989
5,7-DHT	5-HT neurotoxin	CFHB rats (270–300 g)	5 μg	Fornix, 16–20 days	−		Williams et al., 1990
5,7-DHT	5-HT neurotoxin	Wistar rats (180–200 g)	250 μg/10 μl	i.c.v., 1 week before	0		Nazar et al., 1999b
5,7-DHT + Zolpidem (0,1 mg)	5-HT neurotoxin	Wistar rats (180–200 g)	250 μg/10 μl	i.c.v., 1 week before	0	No interaction	Nazar et al., 1999b
5-CT	Non-selective agonist	Lister rats (200–250 g)	1–10 nmol	DPAG	−		Beckett et al., 1992
5-HT	Endogenous ligand	Rats (180–220 g)	10 μg	Nucleus accumbens	−		Plaznik et al., 1991
5-HTP	5-HT precursor	Swiss mice (20–25 g)	50–250	i.p.	0		Wong and Ong, 2001
5-HTP + PCPA (360 mg/kg)	5-HT precursor	Swiss mice (20–25 g)	250	i.p.	+	The combination yielded anxiolytic-like activity	Wong and Ong, 2001
8-OH-DPAT	5-HT_{1A} full agonist	Lister rats (200–250 g)	3–25 nmol	DPAG	−		Beckett et al., 1992
8-OH-DPAT	5-HT_{1A} full agonist	Sprague–Dawley rats (280–320 g)	0.025–0.4	s.c.	−		Ahlenius et al., 1991
8-OH-DPAT	5-HT_{1A} full agonist	Rats (180–220 g)	50–20 μg	Nucleus accumbens	−		Plaznik et al., 1991
8-OH-DPAT	5-HT_{1A} full agonist	CD-COBS rats (200–300 g)	0.125–0.5	s.c.	0	Non-stressed rats	Carli et al., 1989
8-OH-DPAT	5-HT_{1A} full agonist	Wistar rats (180–220 g)	0.0001–0.005	Nucleus accumbens	0		Stefanski et al., 1993
8-OH-DPAT	5-HT_{1A} full agonist	CD-COBS rats (200–300 g)	0.125–0.5	s.c.	+	Stressed rats	Carli et al., 1989
8-OH-DPAT	5-HT_{1A} full agonist	Sprague–Dawley rats (200–250 g)	2.5–5	i.p.	+	Locomotion increased	Lucki et al., 1989
8-OH-DPAT	5-HT_{1A} full agonist	Rats		Hippocampus	+		Plaznik et al., 1991
8-OH-DPAT	5-HT_{1A} full agonist	Wistar rats (180–220 g)	0.025–0.1	i.p.	+	65 dB noise	Stefanski et al., 1992
8-OH-DPAT	5-HT_{1A} full agonist	CD-COBS rats (200–250 g)	0.005	Hippocampus	+		Carli et al., 1993
8-OH-DPAT	5-HT_{1A} full agonist	Wistar rats (180–220 g)	0.0001–0.001	Hippocampus	+		Stefanski et al., 1993
8-OH-DPAT	5-HT_{1A} full agonist	Wistar rats (180–220 g)	0.0005	Hippocampus	+	+5,7-DHT	Stefanski et al., 1993
8-OH-DPAT	5-HT_{1A} full agonist	Wistar rats (175–225 g)	0.03	i.p.	+	Latency to eat in the open field was reduced	Rex et al., 1998
8-OH-DPAT	5-HT_{1A} full agonist	Male and female C57BL6/J×129/sv mice	0.1–1	?	0		Ramboz et al., 1998
Amitriptyline	5-HT reuptake inhibitor	AB mice (4–6 weeks)	5	4 weeks in drinking water	−	Low active mice	Jähkel et al., 1994
Amitriptyline	5-HT reuptake inhibitor	AB mice (4–6 weeks)	5	4 weeks in drinking water	0	High active mice	Jähkel et al., 1994
Amitriptyline	5-HT reuptake inhibitor	Sprague–Dawley rats (325–375 g)	10	i.p., for 21 days (x1)	0		Mar et al., 2000
Amitriptyline	5-HT reuptake inhibitor	Olfactory bulbectomized Sprague–Dawley rats (325–375 g)	10	i.p., for 21 days (x1)	0		Mar et al., 2000
Buspirone	5-HT_{1A} partial agonist	Sprague–Dawley rats (330–420 g)	0.04–10	i.p.	−	15W	Panickar and McNaughton, 1991
Buspirone	5-HT_{1A} partial agonist	Rats (180–220 g)	0.1–5 μg	Nucleus accumbens	−		Plaznik et al., 1991
Buspirone	5-HT_{1A} partial agonist	CD-COBS rats (200–300 g)	0.1–1	s.c.	0	Non-stressed rats	Carli et al., 1989

Buspirone	5-HT_{1A} partial agonist	Wistar rats (180–220 g)	0.0001–0.005	Nucleus accumbens,	0		Stefanski et al., 1993
Buspirone	5-HT_{1A} partial agonist	CD-COBS rats (200–300 g)	0.1–1	s.c.	+	Stressed rats	Carli et al., 1989
Buspirone	5-HT_{1A} partial agonist	Rats		Hippocampus	+		Plaznik et al., 1991
Buspirone	5-HT_{1A} partial agonist	SPRD rats (200 g)	0.62	i.p.	+		Horvath et al., 1992
Buspirone	5-HT_{1A} partial agonist	Wistar rats (180–220 g)	0.3–2.5	i.p.	+	65 dB noise	Stefanski et al., 1992
Buspirone	5-HT_{1A} partial agonist	Rats	0.62–2.5		+		Stefanski et al., 1992
Buspirone	5-HT_{1A} partial agonist	Male and female Wistar rats (180 days)	1.25–2.5	i.p.	+	Sedation?	Hughes, 1993
Buspirone	5-HT_{1A} partial agonist	Wistar rats (180–220 g)	0.0025–0.005	Hippocampus	+		Stefanski et al., 1993
Buspirone	5-HT_{1A} partial agonist	Sprague–Dawley rats (350–650 g)	5	i.p., 5 daily injections	+	Animals were tested on 5 consecutive days	Angrini et al., 1998
Buspirone	5-HT_{1A} partial agonist	Male and female C57BL6/J×129/sv mice	0.05–2.5		0		Ramboz et al., 1998
Buspirone	5-HT_{1A} partial agonist	Wistar rats (180–220 g)	0.3–2.4	i.p.	+	The drug was active on Day 1 and 24 h later after retesting	Siemiatkowski et al., 2000
Buspirone	5-HT_{1A} partial agonist	Sprague–Dawley rats (325–375 g)	3	i.p., for 21 days (x1)	0		Mar et al., 2000
Buspirone	5-HT_{1A} partial agonist	Olfactory bulbectomized Sprague–Dawley rats (325–375 g)	3	i.p., for 21 days (x1)	0		Mar et al., 2000
Citalopram	5-HT reuptake inhibitor	Female Wistar rats (250–350 g)	10–15	i.p.	–		Matto and Allikmets, 1999
Clomipramine	5-HT reuptake inhibitor	AB mice (4–6 weeks)	5	4 weeks in drinking water	0	Low active mice	Jähkel et al., 1994
Clomipramine	5-HT reuptake inhibitor	AB mice (4–6 weeks)	5	4 weeks in drinking water	0	High active mice	Jähkel et al., 1994
Clozapine	Non-selective 5-HT_{2A2C} antagonist	Wistar rats (175–225 g)	1–3	i.p.	+	Latency to eat in the open field was reduced	Rex et al., 1998
Cyanopindolol	Non-selective antagonist	Rats (180–220 g)	0.5 μg	Nucleus accumbens	–		Plaznik et al., 1991
DAU 6215	5-HT_3 antagonist	Crl. CD rats (175–220 g)	0.01–10	p.o.	0		Rizzi et al., 1993
D-Fenfluramine	5-HT stimulant	Female Fischer 344 rats (4 month)	0.6	p.o., for 30–38 days	0		Handa et al., 1996
D-Fenfluramine	5-HT stimulant	Female Fischer 344 rats (21 month)	0.6	p.o., for 30–38 days	0		Handa et al., 1996
Eltoprazine	Non-selective ligand	CD-1 mice (21.1–41.1 g)	1–4	i.p.	+		Kemble et al., 1991
Flesinoxan	5-HT_{1A} full agonist	Sprague–Dawley rats (280–320 g)	0.2–3.2	s.c.	–		Ahlenius et al., 1991
Fluoxetine	5-HT reuptake inhibitor	Female CD1 mice (22–24 g)	5	i.p.	+		De Angelis, 1996
Fluoxetine	5-HT reuptake inhibitor	SHR rats (4–5 weeks old)	5–10	i.p., once a day for 21 days	0	Washout period of 48–51 h	Durand et al., 1999
Fluoxetine	5-HT reuptake inhibitor	Wistar–Kyoto rats (4–5 weeks old)	5–10	i.p., once a day for 21 days	0	Washout period of 48–51 h	Durand et al., 1999
Fluoxetine	5-HT reuptake inhibitor	Sprague–Dawley rats (325–375 g)	10	i.p., for 21 days (x1)	0		Mar et al., 2000
Fluoxetine	5-HT reuptake inhibitor	Olfactory bulbectomized Sprague–Dawley rats (325–375 g)	10	i.p., for 21 days (x1)	0		Mar et al., 2000
Fluoxetine	5-HT reuptake inhibitor	SHR rats (4–5 weeks old)	10	p.o., for 21 days (x1)	0		Durand et al., 2000

(continued on next page)

Table 2 (*continued*)

Drug	Mechanism	Animals	Doses	Routes	Effects	Comments	Reference
Fluoxetine	5-HT reuptake inhibitor	WKY rats (4–5 weeks old)	10	p.o., for 21 days (x1)	0		Durand et al., 2000
Gepirone	5-HT_{1A} partial agonist	Sprague–Dawley rats (441 g)	2.3–4.6	i.p.	–		Knapp et al., 1992
Gepirone	5-HT_{1A} partial agonist	Wistar rats (180–220 g)	0.16–0.62	i.p.	+	65 dB noise	Stefanski et al., 1992
Gepirone	5-HT_{1A} partial agonist	Rats	0.3–0.62		+		Stefanski et al., 1992
Imipramine	NA/5-HT reuptake inhibitor	Female Long–Evans rats (12 weeks)	20	i.p.	–	Saline injection between 6 to 21 days postnatal	Dwyer and Roy, 1993
Imipramine	NA/5-HT reuptake inhibitor	Female Long–Evans rats (12 weeks)	20	i.p., for 11 days (x1)	–	Saline injection between 6 to 21 days postnatal	Dwyer and Roy, 1993
Imipramine	NA/5-HT reuptake inhibitor	Female Long–Evans rats (12 weeks)	20	i.p.	0	No saline injection	Dwyer and Roy, 1993
Imipramine	NA/5-HT reuptake inhibitor	Female Long–Evans rats (12 weeks)	20	i.p., for 11 days (x1)	0	No saline injection	Dwyer and Roy, 1993
Imipramine	5-HT/NA reuptake inhibitor	Female CD1 mice (22–24 g)	10–40	i.p.	0		De Angelis, 1996
Imipramine	5-HT/NA reuptake inhibitor	SHR rats (4–5 weeks old)	10	p.o., for 21 days (x1)	0		Durand et al., 2000
Imipramine	5-HT/NA reuptake inhibitor	WKY rats (4–5 weeks old)	10	p.o., for 21 days (x1)	0		Durand et al., 2000
Ipsapirone	5-HT_{1A} partial agonist	Wistar rats (180–220 g)	0.31–1.25	i.p.	+	65 dB noise	Stefanski et al., 1992
Ipsapirone	5-HT_{1A} partial agonist	Rats	0.3–0.62	?	+		Stefanski et al., 1992
Ipsapirone	5-HT_{1A} partial agonist	Wistar rats (175–225 g)	2	i.p.	+	Latency to eat in the open field was reduced	Rex et al., 1998
Isatin	5-HT stimulant	Wistar mice (25–30 g)	20	i.p.	–		Bhattacharya et al., 1991
Ketanserin	5-HT_2 antagonist	Sprague–Dawley rats (200–250 g)	10	i.p.	0		Lucki et al., 1989
mCPP	5-$HT_{2C/2B}$ agonist	Wistar rats (200–250 g)	1–5	i.p.	–	Sedation?	Klodzinska et al., 1989
mCPP	5-$HT_{2C/2B}$ agonist	Sprague–Dawley rats (200–250 g)	2.5–5	i.p.	–	Locomotion decreased	Lucki et al., 1989
mCPP	5-$HT_{2C/2B}$ agonist	Wistar rats (200–220 g)	0.125–1	i.v.	–		Meert et al., 1997
mCPP	5-$HT_{2C/2B}$ agonist	Wistar rats (200–220 g)	0.63–10	i.p	–		Meert et al., 1997
mCPP	5-$HT_{2C/2B}$ agonist	Wistar rats (200–220 g)	2.5–10	s.c.	–		Meert et al., 1997
mCPP	5-$HT_{2C/2B}$ agonist	Wistar rats (175–225 g)	0.1–3	i.p.	0	Latency to eat in the open field was not modified	Rex et al., 1998
MDMA	5-HT releaser	Charles Foster rats (180–220 g)	5–10	i.p.	–		Bhattacharya et al., 1998
Metergoline	Non-selective antagonist	Sprague–Dawley rats (200–250 g)	0.16–0.62	i.p.	0		Lucki et al., 1989
Methysergide	Non-selective 5-$HT_{2A/2C}$ antagonist	Sprague–Dawley rats (200–250 g)	5–10	i.p.	0		Lucki et al., 1989
Methysergide	Non-selective 5-$HT_{2A/2C}$ antagonist	Rats (180–220 g)	10 μg	Nucleus accumbens	0		Plaznik et al., 1991
Methysergide	Non-selective 5-$HT_{2A/2C}$ antagonist	Wistar rats (200–220 g)	0.63–10	s.c.	0		Meert et al., 1997

Mianserin	5-HT_2 antagonist	Rats Sprague–Dawley (200–250 g)	2.5–5	i.p.	0		Lucki et al., 1989
Mianserin	5-HT_2 antagonist	Wistar rats (200–220 g)	0.63–10	s.c.	0		Meert et al., 1997
Mirtazapine	Non-selective 5-HT antagonist	Wistar rats (200–220 g)	10	s.c.	–		Meert et al., 1997
MK-212	Non-selective agonist	Sprague–Dawley rats (200–250 g)	0.31–0.62	i.p.	–	Locomotion decreased	Lucki et al., 1989
NDO 008	5-HT_{1A} agonist	Rats (180–220 g)	1–5 μg	Nucleus accumbens	0		Płaznik et al., 1991
Ondansetron	5-HT_3 antagonist	Wistar rats (250–270 g)	0.25–20	i.p.	0		Papp and Przegalinski, 1989
Ondansetron	5-HT_3 antagonist	Wistar rats	0.0005–0.005	Hippocampus	0		Stefanski et al., 1993
Ondansetron	5-HT_3 antagonist	Rats		Accumbens	+		Płaznik et al., 1991a
Ondansetron	5-HT_3 antagonist	Wistar rats (180–220 g)	0.1–1.5	i.p.	+	65 dB noise	Stefanski et al., 1992
Ondansetron	5-HT_3 antagonist	Rats	0.001–0.1	?	+		Stefanski et al., 1992
Ondansetron	5-HT_3 antagonist	Wistar rats	0.001–0.0025	Nucleus accumbens septi	+		Stefanski et al., 1993
Ondansetron	5-HT_3 antagonist	Wistar rats (175–225 g)	0.0003	i.p.	+	Latency to eat in the open field was reduced	Rex et al., 1998
Paroxetine	5-HT reuptake inhibitor	Wistar–Kyoto rats	10	i.p., for 10 days (x1)	0		Paré et al., 1999
Paroxetine	5-HT reuptake inhibitor	Sprague–Dawley rats	10	i.p., for 10 days (x1)	0		Paré et al., 1999
Paroxetine	5-HT reuptake inhibitor	Wistar rats	10	i.p., for 10 days (x1)	0		Paré et al., 1999
PCA	5-HT neurotoxin	Wistar rats (286–360 g)	2	i.p., 21 days	0		Harro et al., 2001
PCA + chronic variable stress	5-HT neurotoxin	Wistar rats (286–360 g)	2	i.p., 21 days	0		Harro et al., 2001
PCPA	5-HT synthesis inhibitor	Long–Evans rats (260–300 g)	500–1000	For 2 days (x2, 3 days before testing)	–	Locomotion decreased	Dringenberg et al., 1995
PCPA	5-HT synthesis inhibitor	Sprague–Dawley rats (350–650 g)	100	i.p., 5 daily injections	+	(1) Weak effects; (2) Animals were tested on 5 consecutive days	Angrini et al., 1998
PCPA	5-HT synthesis inhibitor	Wistar rats (180–200 g)	50–300	i.p., twice for 2 days	0		Nazar et al., 1999b
PCPA	5-HT synthesis inhibitor	Wistar rats (180–200 g)	150	i.p., twice for 2 days	0		Nazar et al., 1999b
PCPA + Picrotoxin (0,1 μg)	5-HT synthesis inhibitor	Wistar rats (180–200 g)	150	i.p., twice for 2 days	0	No interaction	Nazar et al., 1999a,b
Pinoline	5-HT reuptake inhibitor	Wistar rats (270–350 g)	15	i.p.	–		Pälikkä et al., 1996
Pizotifen	Non-selective 5-HT antagonist	Wistar rats (200–220 g)	0.63–10	s.c.	0		Meert et al., 1997
Polyclonal anti-5-HT-moduline	Decreases 5-HT release	Swiss mice (28–32 g)	5 μl	i.c.v.	+		Grimaldi et al., 1999
Propranolol	Non-selective 5-HT_{1A} antagonist	Swiss–Webster mice (6–8 weeks)	10	s.c.	–	Latency to emerge after restraint stress	Stone et al., 1995

(continued on next page)

Table 2 (*continued*)

Drug	Mechanism	Animals	Doses	Routes	Effects	Comments	Reference
Propranolol	Non-selective 5-HT_{1A} antagonist	Sprague–Dawley rats (200–250 g)	10	i.p.	+	Locomotion increased	Lucki et al., 1989
Propranolol	Non-selective 5-HT_{1A} antagonist	Sprague–Dawley rats (350–650 g)	5–20	i.p., 5 daily injections	+	(1) The D, L isomer was used; (2) animals were tested on 5 consecutive days	Angrini et al., 1993
Propranolol	Non-selective 5-HT_{1A} antagonist	Wistar rats (175–225 g)	0.3–1	i.p.	+	Latency to eat in the open field was reduced	Rex et al., 1998
Quipazine	Non-selective ligand	Rats (180–220 g)	10–20 μg	Nucleus accumbens	–		Piaznik et al., 1991
R 56413	5-HT_2 antagonist	Rats	0.01–0.63	?	+		Meert and Colpaert, 1986
Ritanserin	5-HT_2 antagonist	Wistar rats (220–240 g)	2.5–10	s.c.	–	Sedation ?	Meert, 1992
Ritanserin	5-HT_2 antagonist	Wistar rats (250–280 g)	0.01–40	s.c.	+		Meert and Colpaert, 1986
Ritanserin	5-HT_2 antagonist	Rats	0.04–10	?	+		Meert and Colpaert, 1986
Ritanserin	5-HT_2 antagonist	Wistar rats (220–240 g)	0.04–0.63	s.c.	+		Meert, 1992
Ritanserin	5-HT_2 antagonist	Wistar rats (180–220 g)	1–5	i.p.	+	65 dB noise	Stefanski et al., 1992
Ritanserin	5-HT_2 antagonist	Rats	5	?	+		Stefanski et al., 1992
Ritanserin	5-HT_2 antagonist	Wistar rats (200–220 g)	0.63–10	s.c.	0		Meert et al., 1997
Ritanserin	5-HT_2 antagonist	Wistar rats (175–225 g)	0.125–0.25	i.p.	+	Latency to eat in the open field was reduced	Rex et al., 1998
TFMPP	Non-selective agonist	Wistar rats (200–250 g)	1–5	i.p.	–	Sedation?	Klodzinska et al., 1989
TFMPP	Non-selective agonist	Sprague–Dawley rats (200–250 g)	2.5–5	i.p.	–	Locomotion decreased	Lucki et al., 1989
Tropisetron	5-HT_3 antagonist	Wistar rats (250–270 g)	0.187–20	i.p.	0		Papp and Przegalinski, 1989
Tropisetron	5-HT_3 antagonist	Wistar rats	0.000001–0.0001	Hippocampus	0		Stefanski et al., 1993
Tropisetron	5-HT_3 antagonist	Rats		Accumbens	+		Piazmik et al., 1991
Tropisetron	5-HT_3 antagonist	Wistar rats (180–220 g)	0.0001–0.01	i.p.	+	65 dB noise	Stefanski et al., 1992
Tropisetron	5-HT_3 antagonist	Rats	0.001–0.1	?	+		Stefanski et al., 1992
Tropisetron	5-HT_3 antagonist	Wistar rats	0.000001–0.00001	Nucleus accumbens septi	+		Stefanski et al., 1993
Tropisetron	5-HT_3 antagonist	Wistar rats	0.000005	Nucleus accumbens septi	+	+5,7-DHT	Stefanski et al., 1993
Tropisetron	5-HT_3 antagonist	Wistar rats (175–225 g)	0.001–0.01	i.p.	+	Latency to eat in the open field was reduced	Rex et al., 1998
WAY 100635	5-HT_{1A} antagonist	Male and female C57BL6/J ×129/sv mice	0.03–0.3		+		Ramboz et al., 1998

+, Anxiolytic-like effect; –, anxiogenic-like effect; 0, no anxiolytic or anxiogenic-like effects (in some cases, non-specific effects can be observed but this will be specified in the "comment" column); 5-HT, 5-hydroxytryptamine (serotonin); DPAG, dorsal periaqueductal gray; i.p., intraperitoneal; p.o., per os; s.c., subcutaneous; i.v., intravenous; i.c.v., intracerebroventricular. Data obtained from G. Griebel, personal database.

example, for chickens, most of the studies report no effect or anxiogenic-like effects after treatment with anxiolytic compounds.

Interestingly, triazolopyridazines such as alprazolam or adinazolam produce effects very different from those of classical benzodiazepine receptor agonists: approximately 1/3 of the studies reported anxiolytic-like effects, 1/3 no effect and 1/3 anxiogenic effects. This variability may be related to the clinical features of these compounds which are well known to be active in anxiety disorders such as for example panic attacks (Uhlenhuth et al., 1989; Westenberg, 1996), rather than on normal anxiety. Indeed, it is to be noted that normal and pathological anxiety have a very different phenomenology and are underlined by very different mechanisms. They are thus modelized in animals by very different situations (Belzung and Griebel, 2001). For example, the mouse defense test battery in mice seem to model certain aspects of panic attacks (Griebel et al., 1995, 1997, 1998), the free exploratory test situation models some aspects some aspects of generalized anxiety (Belzung and Berton, 1997) and exposure of rodents to cat may rather modelize post-traumatic stress disorder (Belzung et al., 2001). One may therefore suggest that the open field test may not be relevant to model such diseases, as it does not offer predictive validity for such disorders. The same poor ability of the open field to detect anxiolytic-like effects of benzodiazepine partial and selective agonists should be noted, which further emphasizes the failure of this procedure to fully mimic the clinical efficacy of the model.

As to benzodiazepine receptor inverse agonists, the situation mirrors that of agonists, as 62.5% of the studies reveal anxiogenic-like effects. Finally, most of the studies on the action of neurosteroid ligands (74%) failed to detect any activity of these compounds. This is one more argument in favor of the idea that the open field may not model various aspects of anxiety disorders, as neurosteroid abnormalities have been specifically detected in some anxiety disorders. For example, patients with generalized anxiety disorder have significantly lower levels of pregnenolone sulfate than do control subjects (Semeniuk et al., 2001).

Finally, 57% of the studies involving $GABA_A$ receptor agonists failed to reveal intrinsic effects of such compounds. This is not very surprising, as GABA receptor agonists are not endowed with anxiolytic activity (Ågmo et al., 1991). The fact that 43% of the studies revealed anxiolytic-like effects with such compounds is surprising and shows that this model may in some cases be sensitive to false positive effects. Barbiturates are generally anxiolytic in the open field (75% of the studies), which parallels clinical data.

3. Effects of serotonin-like acting drugs

Considerable research has been undertaken since the early 1980s on the anxiolytic-like activity of drugs acting on serotonin (5-HT) neurotransmission, particularly compounds that bind selectively on 5-HT_{1A} receptors (agonists, but also antagonists) or inhibit 5-HT reuptake (Selective Serotonin Reuptake Inhibitors) (see Griebel, 1995, 1996, 1999a; Belzung, 2001 for reviews). A summary of the studies investigating the effects of these compounds in animals tested in an open field is presented in Table 2.

Parenteral administration of full or partial agonists of 5-HT_{1A} receptors generally induces anxiolytic-like effects in animals subjected to the open field. Indeed, 8-hydroxydipropylaminotetralin (8-OH-DPAT) elicited anxiolytic-like effects in 62.5% of the studies (exactly as benzodiazepine full agonists) and partial agonists such as buspirone, gepirone or ipsapirone were anxiolytic in 73.3% of the studies. This can be compared to the effects of these compounds in other animal models of anxiety. For example, we have shown that 5-HT_{1A} receptors agonists had anxiolytic-like activity in 74% of the preclinical studies (Belzung, 2001). So, one may conclude that the ability of the open field to detect anxiolysis of 5-HT_{1A} receptors agonists is exactly the same as that of other animal models of anxiety, which renders this model suitable for the assessment of the anxiolytic-like activity of such compounds.

However, this does not extend to other putative anxiolytic treatments. Indeed, chronic administration of not only Selective Serotonin Reuptake Inhibitors (fluoxetine, amitriptyline, clomipramine, paroxetine) but also of other types of antidepressants such as the tricyclic, imipramine, never elicited anxiolytic-like effects. In 89% of the studies, no effects were obtained after such treatments while in some cases anxiogenic actions could be observed. This parallels results obtained with other animal models of anxiety-like behavior. One must remember that the open field test was pharmacologically validated with classical benzodiazepines such as chlordiazepoxide and diazepam that are effective in the treatment of generalized anxiety disorder. In the clinic, chronic Selective Serotonin Reuptake Inhibitors and tricyclics have been used successfully in the treatment of panic attacks (Westenberg, 1996; Wagstaff et al., 2002a,b), social phobia, post-traumatic stress disorder (Wagstaff et al., 2002a,b) and obsessive-compulsive disorders (Thomsen, 2000; Wagstaff et al., 2002a,b), which are anxiety disorders. This further emphasizes that the open field test may not be a model of pathological anxiety, as it has no predictive validity for such disorders.

Regarding the effects of 5-HT_2 receptor antagonists, only ritanserin induced anxiolytic-like effects (75% of the studies) while no effects were observed with other compounds such as ketanserin, methysergide, mianserin or RO 56413. This parallels the classical reports of the high variability of serotonin effects in the clinic. Finally, non-specific 5-HT receptor agonists were always anxiogenic while 5-HT_{1A} receptor antagonists elicited anxiolysis in 56% of the studies and no effect in the other cases. This may be related to differences in mechanisms (some antagonists selectively bind to 5-HT_{1A} receptors while others also have an affinity for other neurotransmitter receptors).

Table 3
Effects of CRF ligands on animals subjected to the open field test

Drug	Mechanism	Animals	Doses (mg/kg)	Routes	Effects	Comments	Reference
α-hel CRF_{9-41}	$CRF_{1/2}$ antagonist	Wistar rats (310–330 g)	5 μg/5 μl	i.c.v.	0		Kumar and Karanth, 1996
α-hel CRF_{9-41}	$CRF_{1/2}$ antagonist	BALB/c mice (10 weeks)	0.8–8 nmol	i.c.v.	0		Moreau et al., 1997
Antisense ODN	CRF gene inhibition	Sprague–Dawley rats (200–250 g)	1 nmol	hippocampus, 4 injections	+	Increased exploration	Wu et al., 1997
CRF	Endogenous peptide	Wistar rats (200–230 g)	0.15 nmol/2 μl	i.c.v.	–		Sutton et al., 1982
CRF	Endogenous peptide	Sprague–Dawley rats (300 g)	150 pmol/2 μl	i.c.v.	–		Britton et al., 1982
CRF	Endogenous peptide	Sprague–Dawley rats (180–230 g)	0.01–1 μg/1 μl	amygdala	–	Decrease in locomotor activity, rearing and hole poking	Liang and Lee, 1988
CRF	Endogenous peptide	BALB/c mice (20–25 g)	0.01 μg/0.4 μl	dendate gyrus of hippocampus	–	Increased locomotor activity in the center	Lee and Tsai, 1989
CRF	Endogenous peptide	BALB/c mice (20–25 g)	0.02 μg/0.5 μl	amygdala	–	Increased locomotor activity in the center	Lee and Tsai, 1989
CRF	Endogenous peptide	Sprague–Dawley rats (250 g)	60 pmol/2 μl	i.c.v.	–		Britton and Indyk, 1996
CRF	Endogenous peptide	Wistar rats (310–330 g)	0.1–0.4 μg/5 μl	i.c.v.	–		Kumar and Karanth, 1996
CRF	Endogenous peptide	BALB/c mice (20–25 g)	0.2 μg/2 μl	i.c.v.	–	Increased center region activity	Lee et al., 1987
CRF	Endogenous peptide	Wistar rats (200–230 g)	0.015–7.5 nmol/2 μl	s.c.	0		Sutton et al., 1982
CRF	Endogenous peptide	BALB/c mice (20–25 g)	0.05 μg/0.7 μl	caudate nucleus	0		Lee and Tsai, 1989
CRF + α-hel CRF_{9-41} (5 μg/5 μl)	Endogenous peptide	Wistar rats (310–330 g)	0.1–0.4 μg	i.c.v.	(+)		Kumar and Karanth, 1996
CRF + Diazepam (2 mg/kg)	Endogenous peptide	BALB/c mice (20–25 g)	0.2 μg/2 μl	i.c.v.	(+)		Lee et al., 1987
Urocortin	Endogenous CRF_2 ligand	BALB/c mice (10 weeks)	0.06 nmol	i.c.v.	–		Moreau et al., 1997
Urocortin + α-hel CRF_{9-41} (2.6–8 nmol)	Endogenous CRF_2 ligand	BALB/c mice (10 weeks)	0.06 nmol	i.c.v.	(+)		Moreau et al., 1997
Urocortin + Diazepam (0.1–1)	Endogenous CRF_2 ligand	BALB/c mice (10 weeks)	0.06 nmol	i.c.v.	(+)		Moreau et al., 1997
Antisense ODN + CRF (0.5 μg)	Blockade of CRF_1 receptor translation	Wistar rats (200–250 g)	0.5 μl/h	minipumps, 3 days	(+)		Skutella et al., 1998
CRF	Endogenous peptide	Wistar rats (200–250 g)	0.5 μg	i.c.v.	–		Skutella et al., 1998
Urocortin + CRF-OH	Endogenous CRF_2 ligand	Rats	0.1 μg	i.c.v.	0		Zorrilla et al., 1998
Urocortin + CRF_{6-33}	Endogenous CRF_2 ligand	Rats	0.1 μg	i.c.v.	0		Zorrilla et al., 1998

+, Anxiolitic-like effect; –, anxiogenic-like effect; 0, no anxiolytic or anxiogenic-like effects (in some cases, non-specific effects can be observed but this will be specified in the "comment" column); (+) antagonism of anxiogenic-like effects; (–), antagonism of anxiolytic-like effects; s.c., subcutaneous; i.c.v., intracerebroventricular. Data obtained from G. Griebel, personal database.

Finally, in situ administration of 5-HT_{1A} receptor agonists in limbic structures such as the hippocampus was always anxiolytic. This parallels the effects observed after stimulation of the 5-HT_{1A} pre-synaptic receptors in other rodent models of anxiety (see Griebel, 1995 for a review) and suggests that the anxiolytic activity of 5-HT_{1A} receptors may be related to a pre-synaptic target.

4. Effects of neuropeptide receptor ligands

Recently, the rapid advances in neuropeptide research have stimulated interest in the ability of some neuropeptides to act as anxiolytics (see Griebel, 1999b for an excellent review). Interest has focused on CRF receptor ligands, on cholecystokinin, neuropeptide Y, tachykinin (especially neurokinin) as well as on glucocorticoid and mineralocorticoid receptor ligands.

Studies investigating the effects of CRF receptor ligands are presented in Table 3. This table shows clearly that all the studies involving i.c.v. injections of CRF found anxiogenic effects of the neuropeptide. The sole study that found no intrinsic activity had assessed the effects of s.c. injected CRF so that the failure of CRF to induce anxiogenesis may be attributed to rapid degradation of the peptide. All these anxiogenic effects may be due to the interaction of CRF with molecular targets situated in the limbic structures as anxiogenic effects were obtained after intra-amygdala and intra-hippocampal injections of the peptide. Unfortunately, no study investigated the effects of specific CRF receptor antagonists, so that it is very difficult to conclude further about the ability of the open field test to detect anxiolytic activity of such ligands.

Data for the effects of other neuropeptide receptor ligands are in Table 4. We found only 15 studies describing such effects, 9 of which concerned the effects of neuro-

Table 4
Effects of non-CRF neuropeptide ligand on animals subjected to the open field test

Drug	Mechanism	Animals	Doses	Routes	Effects	Comments	Reference
ANP	Neuropeptide	Wistar rats (180–200 g)	200–500 ng/5 μl	i.c.v.	+		Bhattacharya et al., 1996
Atriopeptin II	Residue peptide	Wistar rats (220–260 g)	5–10 μg/rat	i.c.v.	+		Poggioli et al., 1992
BIBP3226	Y_1 antagonist	Wistar rats (280–350 g)	0.5 μg	DPAG	0		Kask et al., 1998
BIBP3226	Y_1 antagonist	Wistar rats (280–350 g)		i.c.v.	0		Kask et al., 1998
BIBP3226	Y_1 antagonist	Wistar rats (300–450 g)	5 μg/6.5 μl	i.c.v.	0		Kask et al., 1998
BIBP3226	Y_1 antagonist	Wistar rats (300–450 g)	0.5 μg/6.5 μl	DPAG	0		Kask et al., 1998
CCK-8s	CCK1/B agonist	Sprague–Dawley rats (200–220 g)	100 pmol/1 μl	median nucleus accumbens	–		Daugé et al., 1989
CCK-8s	CCK1/B agonist	Sprague–Dawley rats (200–220 g)	1 fmol–100 pmol/1 μl	median nucleus accumbens	0	Rats were habituated to the environment	Daugé et al., 1989
CGP71683A	Y_5 antagonist	Wistar rats (280–350 g)	10	i.p.	–	Rats exposed to the elevated plus maze before open field testing	Kask et al., 2001
GR 64349	NK_2 agonist	Rats	100–1000 pmol	dorsal raphé	–		Stratton et al., 1993
Neuropeptide Y	Endogenous peptide	Sprague–Dawley rats (220–250 g)	1–4 nmol/5 μl	i.c.v.	?	NPY decreased spontaneous activity	Heilig and Murison, 1987
RU28318	Mineralocorticoid antagonist	Long–Evans rats (300–400 g)	0.5 ng/0.5 μl	hippocampus	+		Bitran et al., 1998
RU28318 + Dexamethasone	Mineralocorticoid antagonist	Long–Evans rats (300–400 g)	0.5 ng/0.5 μl	hippocampus	(0)		Bitran et al., 1998
RU38486	Glucocorticoid antagonist	Long–Evans rats (300–400 g)	0.2–0.5 ng/ 0.5 μl	hippocampus	0		Bitran et al., 1998
RU38486 + Dexamethasone	Glucocorticoid antagonist	Long–Evans rats (300–400 g)	0.2–0.5 ng/ 0.5 μl	hippocampus	0		Bitran et al., 1998

+, Anxiolytic-like effect; –, anxiogenic-like effect; 0, no anxiolytic or anxiogenic-like effects (in some cases, non-specific effects could be observed but this will be specified in the "comment" column); (0) no antagonism; i.p., intraperitoneal; i.c.v., intracerebroventricular; DPAG, dorsal periaqueductal gray. Data obtained from G. Griebel, personal database.

peptide injected directly into some specific brain areas. Most of the studies concerned the effects of neuropeptide Y ligands, either neuropeptide Y Y1 receptor antagonists (BIBP3226) or neuropeptide Y Y5 receptor antagonist (CGP71683A). The neuropeptide Y Y1 receptor antagonist, administered parenterally or within the dorsal periaqueductal gray, never elicited any intrinsic action while the neuropeptide Y Y5 receptor antagonist was anxiogenic. The glucorticoid receptor antagonist did not elicit any effect when injected within the hippocampus. In fact, due to the scarcity of data, it is very difficult to reach a relevant way conclusion.

We applied the Griebel (1999b) synthesis to the preclinical studies investigating the effects of neuropeptide ligands in animal models of anxiety to calculate the proportion of articles using the open field. Surprisingly, very few used this paradigm: only 2/359 studies investigating the effects of CCK ligands, 17/343 articles investigating the effects of CRF receptors ligands; 1/51 interested in the action of NPY ligands and 1/52 in the articles studying the effects of neurokinin receptor ligands. The other studies were done with other animal models of anxiety, such as the elevated plus maze, the light–dark boxes, the mouse defense test battery or more classical conditioned conflict tests. Why? Two hypotheses can be suggested: either the open field test is no longer up-to-date (because of the availability of other tests), or was used and negative results were obtained that were not found relevant for publication. As to the first hypothesis, one may argue that this is not very probable. Indeed, in a recent review on the genetics of anxiety-like behavior in rodent models (Clement et al., 2002), we showed that the open field test was used in 30/68 studies. These studies were mostly very recent. Therefore, one may propose that neuropeptides may not have very marked anxiolytic-like effects in the open field.

5. Conclusion

Is the open field test suitable for screening anxiolytic activity of pharmacological treatments? To be a relevant model of human behavior, an animal test should fit three criteria: predictive, face and construct validity. Our review of the literature shows clearly that the open field cannot claim predictive validity for anxiety in general, as it is not sensitive to compounds (alprazolam and chronic Selective Serotonin Reuptake Inhibitors) effective in anxiety disorders such as panic, obsessional compulsive disorder, social phobias and post-traumatic stress disorder. In fact, it seems to be sensitive only to the anxiolytic effects produced by classical benzodiazepines and 5-HT_{1A} receptor agonists. Therefore, one may suggest that the open field may either be a model of the normal anxiety everyone is faced by when confronted with a stressful or threatening situation but not with the features of pathological anxiety or, alternatively, it may be a model to test the behavioral effects of classical benzodiazepines and 5-HT_{1A} agonists. However, a radical-seeming cautionary comment is needed here. Indeed, the disorders termed "Anxiety disorders" in the DSM-IV (1994) are called "Somatoform, stress-related and neurotic disorders" in the ICD-10 (1994) classification of the World Health Organization. In fact, it is possible that we are trapped by the terminology and that the psychiatric diseases termed "anxiety disorders" may have no relationship with anxiety-like behavior. In this case, of course, the open field may gain in predictive validity.

As to the face and construct validity of this model, one may propose that they are fulfilled. In fact, face validity implies that the anxiety response (the phenomenological aspect) observed in the animal is identical to the one observed in humans. In the open field, the observed behavior is avoidance of threatening places, which can also be observed in humans. In rodents, forced confrontation with novelty is stressful (Misslin and Cigrang, 1986). Stress induces anxiety-like behaviors, as it does in humans. So, the model may also fit construct validity (similar etiology).

In conclusion, the open field test may be a rodent model of normal anxiety, sensitive to the anxiolytic-like effects of classical benzodiazepines and 5-HT_{1A} receptor agonists but not to the effects of compounds displaying anxiolytic-like effects in the clinical entity termed "anxiety disorders".

Acknowledgements

The authors are thankful to Dr. G. Griebel for providing data from his personal database (Tables 2, 3 and 4).

References

Adams, P.M., 1982. Effects of perinatal chlordiazepoxide exposure on rat preweaning and postweaning behavior. Neurobehav. Toxicol. Teratol. 4, 279–282.

Ågmo, A., Pruneda, R., Guzman, M., Gutierrez, M., 1991. GABAergic drugs and conflict behavior in the rat: lack of similarities with the actions of benzodiazepines. Naunyn-Schmiedeberg's Arch. Pharmacol. 344, 314–322.

Ahlenius, S., Larsson, K., Wijkstrom, A., 1991. Behavioral and biochemical effects of the 5-HT_{1A} receptor agonists flesinoxan and 8-OH-DPAT in the rat. Eur. J. Pharmacol. 200, 259–266.

Alleva, E., Laviola, G., Tirelli, E., Bignami, G., 1985. Short-, medium-, and long-term effects of prenatal oxazepam on neurobehavioural development of mice. Psychopharmacology 87, 434–441.

Andersen, I., Faerevik, G., Boe, K.E., Janczak, A.M., Bakken, M., 2000. Effects of diazepam on the behavior of weaned pigs in three putative models of anxiety. Appl. Anim. Behav. Sci. 68, 121–130.

Angrini, M., Leslie, J.C., Shephard, R.A., 1998. Effects of propranolol, buspirone, pCPA, reserpine, and chlordiazepoxide on open-field behavior. Pharmacol. Biochem. Behav. 59, 387–397.

Barros, H.M., Tannhauser, S.L., Tannhauser, M.A., Tannhauser, M., 1994. The effects of GABAergic drugs on grooming behaviour in the open field. Pharmacol. Toxicol. 74, 339–344.

Bast, T., Zhang, W.N., Feldon, J., 2001a. Hyperactivity, decreased startle reactivity, and disrupted prepulse inhibition following disinhibition of

the rat ventral hippocampus by the GABA(A) receptor antagonist picrotoxin. Psychopharmacology (Berl.) 156, 225–233.

Bast, T., Zhang, W.N., Feldon, J., 2001b. The ventral hippocampus and fear conditioning in rats. Different anterograde amnesias of fear after tetrodotoxin inactivation and infusion of the GABA(A) agonist muscimol. Exp. Brain Res. 139, 39–52.

Beaufour, C.C., Le Bihan, C., Hamon, M., Thiebot, M., 2001. Extracellular dopamine in the rat prefrontal cortex during reward-, punishment- and novelty-associated behaviour. Effects of diazepam. Pharmacol. Biochem. Behav. 69, 133–412.

Beckett, S.R., Lawrence, A.J., Marsden, C.A., Marshall, P.W., 1992. Attenuation of chemically induced defence response by 5-HT1 receptor agonists administered into the periaqueductal gray. Psychopharmacology (Berl.) 108, 104–110.

Belzung, C., 1999. Measuring exploratory behavior. In: Crusio, W.E., Gerlai, R.T. (Eds.), Handbook of Molecular Genetic Techniques for Brain and Behavior Research (Techniques in the Behavioral and Neural Sciences). Elsevier, Amsterdam, pp. 739–749.

Belzung, C., 2001. Rodent models of anxiety-like behaviors: are they predictive for compounds acting via non-benzodiazepine mechanisms? Curr. Opin. Investig. Drugs 2, 1108–1111.

Belzung, C., Berton, F., 1997. Further pharmacological validation of the Balb/c neophobia in the free exploratory paradigm as an animal model of trait anxiety. Behav. Pharmacol. 8, 541–548.

Belzung, C., Griebel, G., 2001. Measuring normal and pathological anxiety-like behaviour in mice: a review. Behav. Brain Res. 125, 141–149.

Belzung, C., El Hage, W., Moindrot, N., Griebel, G., 2001. Behavioral and neurochemical changes following predatory stress in mice. Neuropharmacology 41, 400–408.

Belzung, C., Griebel, G., Dubois-Carmagnat, F., Darves-Bornoz, J.M., 2002. Amino acid transmitter systems. In: D'Haenen, H., den Boer, J.A., Willner, P. (Eds.), Biological Psychiatry. Wiley, Chichester, England, pp. 915–927.

Bert, B., Fink, H., Sohr, R., Rex, A., 2001. Different effects of diazepam in Fischer rats and two stocks of Wistar rats in tests of anxiety. Pharmacol. Biochem. Behav. 70, 411–420.

Bhattacharya, S.K., Clow, A., Przyborowska, A., Halket, J., Glover, V., Sandler, M., 1991. Effect of aromatic amino acids, pentylenetetrazole and yohimbine on isatin and tribulin activity in rat brain. Neurosci. Lett. 132, 44–46.

Bhattacharya, S.K., Sen, A.P., Upadhyay, S.N., Jaiswal, A.K., 1993. Anxiolytic activity of piracetam, a nootropic agent, following subchronic administration in rodents. Indian J. Exp. Biol. 31, 902–907.

Bhattacharya, S.K., Chakrabarti, A., Sandler, M., Glover, V., 1996. Anxiolytic activity of intraventricularly administered atrial natriuretic peptide in the rat. Neuropsychopharmacology 15, 199–206.

Bhattacharya, S.K., Bhattacharya, A., Ghosal, S., 1998. Anxiogenic activity of methylenedioxymethamphetamine (Ecstasy): an experimental study. Biog. Amine. 14, 217–237.

Bitran, D., Shiekh, M., Dowd, J.A., Dugan, M.M., Renda, P., 1998. Corticosterone is permissive to the anxiolytic effect that results from the blockade of hippocampal mineralocorticoid receptors. Pharmacol. Biochem. Behav. 60, 879–887.

Boerngen-Lacerda, R., Souza-Formigoni, M.L., 2000. Does the increase in locomotion induced by ethanol indicate its stimulant or anxiolytic properties? Pharmacol. Biochem. Behav. 67, 225–232.

Borsini, F., Giuliani, S., Meli, A., 1988. Functional responses to baclofen and 4,5,6,7-tetrahydroisoxazolo (5,4-*c*) pyridin-3-ol (THIP) in rats repeatedly treated with desipramine. Pharmacol. Biochem. Behav. 29, 189–191.

Britton, D.R., Britton, K.T., 1981. A sensitive open field measure of anxiolytic drug activity. Pharmacol. Biochem. Behav. 15, 577–582.

Britton, D.R., Indyk, E., 1990. Central effects of corticotropin releasing factor (CRF): evidence for similar interactions with environmental novelty and with caffeine. Psychopharmacology (Berl.) 101, 366–370.

Britton, D.R., Koob, G.F., Rivier, J., Vale, W., 1982. Intraventricular corticotropin-releasing factor enhances behavioral effects of novelty. Life Sci. 31, 363–367.

Broderick, P.A., Hope, O., Jeannot, P., 1998. Mechanism of triazolobenzodiazepine and benzodiazepine action in anxiety and depression: behavioral studies with concomitant in vivo CA1 hippocampal norepinephrine and serotonin release detection in the behaving animal. Prog. Neuropsychopharmacol. Biol. Psychiatry 22, 353–386.

Bruhwyler, J., 1990. Anxiolytic potential of a microgram dose of chlordiazepoxide in the open-field test. Eur. J. Pharmacol. 187, 547–549.

Bruhwyler, J., Chleide, E., Houbeau, G., Mercier, M., 1991. Stimulant effect of the beta-carboline FG 7142 in the open-field test. Eur. J. Pharmacol. 200, 183–185.

Bruijnzeel, A.W., Stam, R., Wiegant, V.M., 2001. Effect of a benzodiazepine receptor agonist and corticotropin-releasing hormone receptor antagonists on long-term foot-shock-induced increase in defensive withdrawal behavior. Psychopharmacology (Berl.) 158, 132–139.

Cannizzaro, C., Martire, M., Cannizzaro, E., Provenzano, G., Gagliano, M., Carollo, A., Mineo, A., Steardo, L., 2001. Long-lasting handling affects behavioural reactivity in adult rats of both sexes prenatally exposed to diazepam. Brain Res. 904, 225–233.

Car, H., Kuziemka, L., Eska, M., Wisniewski, K., 1996. The NMDA and GABA-A receptors in behavioral activity of rats. Acta Physiol. Hung. 84, 269–270.

Carli, M., Prontera, C., Samanin, R., 1989. Effect of 5-HT1A agonists on stress-induced deficit in open field locomotor activity of rats: evidence that this model identifies anxiolytic-like activity. Neuropharmacology 28, 471–476.

Carli, M., Tatarczynska, E., Cervo, L., Samanin, R., 1993. Stimulation of hippocampal 5-HT1A receptors causes amnesia and anxiolytic-like but not antidepressant-like effects in the rat. Eur. J. Pharmacol. 234, 215–221.

Chesley, S., Lumpkin, M., Schatzki, A., Galpern, W.R., Greenblatt, D.J., Shader, R.I., Miller, L.G., 1991. Prenatal exposure to benzodiazepine: I. Prenatal exposure to lorazepam in mice alters open-field activity and GABAA receptor function. Neuropharmacology 30, 53–58.

Choleris, E., Thomas, A.W., Kavaliers, M., Prato, F.S., 2001. A detailed ethological analysis of the mouse open field test: effects of diazepam, chlordiazepoxide and an extremely low frequency pulsed magnetic field. Neurosci. Biobehav. Rev. 25, 235–260.

Clement, Y., Calatayud, F., Belzung, C., 2002. Genetic basis of anxiety-like behaviour: a critical review. Brain Res. Bull. 57, 57–71.

Coscina, D.V., Nobrega, J.N., 1989. Effects of lateral hypothalamic lesions on the anorexia induced by ethanolamine-*O*-sulfate. Pharmacol. Biochem. Behav. 32, 275–281.

Crawley, J.N., 1981. Neuropharmacologic specificity of a simple animal model for the behavioral actions of benzodiazepines. Pharmacol. Biochem. Behav. 15, 695–699.

Czlonkowska, A.I., Sienkiewicz-Jarosz, H., Siemiatkowski, M., Bidzinski, A., 1999. The effects of neurosteroids on rat behavior and 3H-muscimol binding in the brain. Pharmacol. Biochem. Behav. 63, 639–646.

Daugé, V., Dor, A., Feger, J., Roques, B.P., 1989. The behavioral effects of CCK8 injected into the medial nucleus accumbens are dependent on the motivational state of the rat. Eur. J. Pharmacol. 163, 25–32.

De Angelis, L., 1996. Experimental anxiety and antidepressant drugs: the effects of moclobemide, a selective reversible MAO-A inhibitor, fluoxetine and imipramine in mice. Naunyn-Schmiedeberg's Arch. Pharmacol. 354, 379–383.

De Angelis, L., Bertolissi, M., Nardini, G., Traversa, U., Vertua, R., 1982. Interaction of caffeine with benzodiazepines: behavioral effects in mice. Arch. Int. Pharmacodyn. Ther. 255, 89–102.

Delini-Stula, A., Hunn, C., 1988. Differential effects of anxiolytics and beta-receptor blocking drugs on novelty-oriented ("neophobic") behavior in the rat. Pharmacopsychiatry 21, 186–191.

Depaulis, A., Vergnes, M., 1986. Elicitation of intraspecific defensive behaviors in the rat by microinjection of picrotoxin, a gamma-aminobutyric acid antagonist, into the midbrain periaqueductal gray matter. Brain Res. 367, 87–95.

De Souza Spinosa, H., Gerenutti, M., Bernardi, M.M., 2000. Anxiolytic and anticonvulsant properties of doramectin in rats: behavioral and neurochemistric evaluations. Comp. Biochem. Physiol., Toxicol. Pharmacol. 127, 359–366.

Dringenberg, H.C., Hargreaves, E.L., Baker, G.B., Cooley, R.K., Vanderwolf, C.H., 1995. *p*-Chlorophenylalanine-induced serotonin depletion: reduction in exploratory locomotion but no obvious sensory-motor deficits. Behav. Brain Res. 68, 229–237.

DSM-IV, 1994. Diagnostic and Statistical Manual of Mental Disorders, 4th ed. American Pyschiatric Association, Washington, DC.

Durand, M., Berton, O., Aguerre, S., Edno, L., Combourieu, I., Mormede, P., Chaouloff, F., 1999. Effects of repeated fluoxetine on anxiety-related behaviours, central serotonergic systems, and the corticotropic axis in SHR and WKY rats. Neuropharmacology 38, 893–907.

Durand, M., Aguerre, S., Fernandez, F., Edno, L., Combourieu, I., Mormede, P., Chaouloff, F., 2000. Strain-dependent neurochemical and neuroendocrine effects of desipramine, but not fluoxetine or imipramine, in spontaneously hypertensive and Wistar–Kyoto rats. Neuropharmacology 39, 2464–2477.

Dwyer, K.D., Roy, E.J., 1993. Juvenile desipramine reduces adult sensitivity to imipramine in two behavioral tests. Pharmacol. Biochem. Behav. 45, 201–217.

Fahey, J.M., Pritchard, G.A., Grassi, J.M., Pratt, J.S., Shader, R.I., Greenblatt, D.J., 2001. Pharmacodynamic and receptor binding changes during chronic lorazepam administration. Pharmacol. Biochem. Behav. 69, 1–8.

Fahey, J.M., Pritchard, G.A., Pratt, J.S., Shader, R.I., Greenblatt, D.J., 1999. Lorazepam attenuates the behavioral effects of dizocilpine. Pharmacol. Biochem. Behav. 62, 103–110.

Fernandez-Teruel, A., Escorihuela, R.M., Boix, F., Tobena, A., 1990. Picrotoxin changes the effects of imipramine and desipramine in rats in the forced swimming test. Eur. J. Pharmacol. 181, 35–41.

Ferre, P., Escorihuela, R.M., Tobena, A., Fernandez-Teruel, A., 1996. Evaluation of perinatal flumazenil effects on the behavior of female RLA/Verh rats in anxiety tests and shuttle box avoidance. Pharmacol. Biochem. Behav. 55, 475–480.

File, S.E., Tucker, J.C., 1983. Lorazepam treatment in the neonatal rat alters submissive behavior in adulthood. Neurobehav. Toxicol. Teratol. 5, 289–294.

Fiore, M., Dell'Omo, G., Alleva, E., Lipp, H.P., 1995. A comparison of behavioural effects of prenatally administered oxazepam in mice exposed to open-fields in the laboratory and the real world. Psychopharmacology (Berl.) 122, 72–77.

Fonseca, N.M., Sell, A.B., Carlini, E.A., 1976. Differential behavioral responses of male and female adult rats treated with five psychotropic drugs in the neonatal stage. Psychopharmacologia 46, 263–268.

Frye, C.A., Lacey, E.H., 1999. The neurosteroids DHEA and DHEAS may influence cognitive performance by altering affective state. Physiol. Behav. 66, 85–92.

Frye, C.A., Sturgis, J.D., 1995. Neurosteroids affect spatial/reference, working, and long-term memory of female rats. Neurobiol. Learn. Mem. 64, 83–96.

Gai, N., Grimm, V.E., 1982. The effect of prenatal exposure to diazepam on aspects of postnatal development and behavior in rats. Psychopharmacology 78, 225–229.

Galpern, W.R., Lumpkin, M., Greenblatt, D.J., Shader, R.I., Miller, L.G., 1991. Chronic benzodiazepine administration: VII. Behavioral tolerance and withdrawal and receptor alterations associated with clonazepam administration. Psychopharmacology 104, 225–230.

Garcia-Gil, L., De Miguel, R., Romero, J., Perez, A., Ramos, J.A., Fernandez-Ruiz, J.J., 1999. Perinatal delta9-tetrahydrocannabinol exposure augmented the magnitude of motor inhibition caused by GABA(B), but not GABA(A), receptor agonists in adult rats. Neurotoxicol. Teratol. 21, 277–283.

Gentsch, C., Lichteiner, M., Feer, H., 1989. Behavioral effects of yohimbine and chlordiazepoxide: dependence on the rat's previous familiarization with the test conditions. Neuropsychobiology 22, 101–107.

Griebel, G., 1995. 5-Hydroxytryptamine-interacting drugs in animal models of anxiety disorders: more than 30 years of research. Pharmacol. Ther. 65, 319–395.

Griebel, G., 1996. Variability in the effects of 5-HT-related compounds in experimental models of anxiety: evidence for multiple mechanisms of 5-HT in anxiety or never ending story? Pol. J. Pharmacol. 48, 129–136.

Griebel, G., 1999a. 5-HT_{1A} receptor blockers as potential drug candidates for the treatment of anxiety disorders. Drug News Perspect. 12, 484–490.

Griebel, G., 1999b. Is there a future for neuropeptide receptor ligands in the treatment of anxiety disorders? Pharmacol. Ther. 82, 1–61.

Griebel, G., Blanchard, D.C., Jung, A., Lee, J.C., Masuda, C.K., Blanchard, R.J., 1995. Further evidence that the mouse defense test battery is useful for screening anxiolytic and panicolytic drugs: effects of acute and chronic treatment with alprazolam. Neuropharmacology 34, 1625–1633.

Griebel, G., Perrault, G., Sanger, D.J., 1997. Behavioural profiles of the reversible monoamine-oxidase-A inhibitors befloxatone and moclobemide in an experimental model for screening anxiolytic and anti-panic drugs. Psychopharmacology 131, 180–186.

Griebel, G., Curet, O., Perrault, G., Sanger, D.J., 1998. Behavioral effects of phenelzine in an experimental model for screening anxiolytic and anti-panic drugs: correlation with changes in monoamine-oxidase activity and monoamine levels. Neuropharmacology 37, 927–935.

Grimaldi, B., Bonnin, A., Fillion, M.P., Prudhomme, N., Fillion, G., 1999. 5-Hydroxytryptamine-moduline: a novel endogenous peptide involved in the control of anxiety. Neuroscience 93, 1223–1225.

Guillamon, A., Cales, J.M., Rodriguez-Zafra, M., Perez-Laso, C., Caminero, A., Izquierdo, M.A., Segovia, S., 1990. Effects of perinatal diazepam administration on two sexually dimorphic nonreproductive behaviors. Brain Res. Bull. 25, 913–916.

Hall, C.S., 1934. Emotional behavior in the rat: I. Defecation and urination as measures of individual differences in emotionality. J. Comp. Psychol. 18, 385–403.

Handa, R.J., George, M., Gordon, B.H., Campbell, D.B., Lorens, S.A., 1996. Responses to novelty stress in female F344 rats: effects of age and D-fenfluramine treatment. Pharmacol. Biochem. Behav. 53, 641–664.

Hard, E., Engel, J., Larsson, K., Musi, B., 1985. Effect of diazepam, apomorphine and haloperidol on the audiogenic immobility reaction and on the open field behavior. Psychopharmacology 85, 106–110.

Harro, J., Tonissaar, M., Eller, M., Kask, A., Oreland, L., 2001. Chronic variable stress and partial 5-HT denervation by parachloroamphetamine treatment in the rat: effects on behavior and monoamine neurochemistry. Brain Res. 899, 227–239.

Heilig, M., Murison, R., 1987. Intracerebroventricular neuropeptide Y suppresses open field and home cage activity in the rat. Regul. Pept. 19, 221–231.

Hiltunen, A.J., Jarbe, T.U., 1986. Discrimination of Ro 11-6896, chlordiazepoxide and ethanol in gerbils: generalization and antagonism tests. Psychopharmacology 89, 284–290.

Horvath, K., Andrasi, F., Botka, P., Hamori, T., 1992. Anxiolytic profile of girisopam and GYKI 52,322 (EGIS 6775). Comparison with chlordiazepoxide and buspirone. Acta Physiol. Hung. 79, 153–161.

Hughes, R.N., 1993. Effects on open-field behavior of diazepam and buspirone alone and in combination with chronic caffeine. Life Sci. 53, 1217–1225.

Huttunen, P., Myers, R.D., 1986. Tetrahydro-beta-carboline micro-injected into the hippocampus induces an anxiety-like state in the rat. Pharmacol. Biochem. Behav. 24, 1733–1738.

ICD-10, 1994. International Statistical Classification of Diseases and Related Health Problems (10th Revision). WHO Publications.

Jähkel, M., Oehler, J., Schumacher, H.E., 1994. Influence of nootropic and antidepressive drugs on open field and running wheel behavior in spontaneously high and low active mice. Pharmacol. Biochem. Behav. 49, 263–269.

Jarbe, T.U., Johansson, J.O., 1977. Pentobarbital, diazepam and bemegride:

their effects on open-field behavior in the gerbil (Meriones unguiculatus). Arch. Int. Pharmacodyn. Ther. 225, 88–97.

June, H.L., Lewis, M.J., 1989. Ro15-4513 enhances and attenuates motor stimulant effects of ethanol in rats. Alcohol 6, 245–248.

June, H.L., Johnson, L.T., Lewis, M.J., 1989. Ro15-4513 antagonizes depression of open-field horizontal activity by ethanol in rats. Alcohol 6, 335–337.

June, H.L., Cason, C.R., Cheatham, G., Lui, R., Gan, T., Cook, J.M., 1998. GABAA-benzodiazepine receptors in the striatum are involved in the sedation produced by a moderate, but not an intoxicating ethanol dose in outbred Wistar rats. Brain Res. 794, 103–118.

Kask, A., Rago, L., Harro, J., 1998. Anxiogenic-like effect of the NPY Y1 receptor antagonist BIBP3226 administered into the dorsal periaqueductal gray matter in rats. Regul. Pept. 75–76, 255–262.

Kask, A., Vasar, E., Heidmets, L.T., Allikmets, L., Wikberg, J.E., 2001. Neuropeptide Y Y(5) receptor antagonist CGP71683A: the effects on food intake and anxiety-related behavior in the rat. Eur. J. Pharmacol. 414, 215–224.

Kemble, E.D., Gibson, B.M., Rawleigh, J.M., 1991. Effects of eltoprazine hydrochloride on exploratory behavior and social attraction in mice. Pharmacol. Biochem. Behav. 38, 759–762.

Khisti, R.T., Chopde, C.T., Jain, S.P., 2000. Antidepressant-like effect of the neurosteroid 3alpha-hydroxy-5alpha-pregnan-20-one in mice forced swim test. Pharmacol. Biochem. Behav. 67, 137–143.

Klodzinska, A., Jaros, T., Chojnacka-Wojcik, E., Maj, J., 1989. Exploratory hypoactivity induced by *m*-trifluoromethylphenylpiperazine (TFMPP) and *m*-chlorophenylpiperazine (*m*-CPP). J. Neural Transm., Parkinson's Dis. Dement. Sect. 1, 207–218.

Knapp, D.J., Benjamin, D., Pohorecky, L.A., 1992. Effects of gepirone on ethanol consumption, exploratory behavior, and motor performance in rats. Drug Dev. Res. 26, 319–341.

Kopp, C., Misslin, R., Vogel, E., Rettori, M.C., Delagrange, P., Guardiola-Lemaître, B., 1997. Effects of day-length variations on emotional responses toward unfamiliarity in Swiss mice. Behav. Proc. 41, 151–157.

Kumar, K.B., Karanth, K.S., 1996. Alpha-helical CRF blocks differential influence of corticotropin releasing factor (CRF) on appetitive and aversive memory retrieval in rats. J. Neural Transm. 103, 1117–1126.

Lambadjieva, N.D., 1998. Influence of testosterone on some behavioral reactions of male immature rats. Methods Find. Exp. Clin. Pharmacol. 20, 673–677.

Lambadjieva, N.D., 1999. Influence of testosterone on some behavioral reactions of male immature rats exposed to continual light. Methods Find. Exp. Clin. Pharmacol. 21, 17–20.

Laviola, G., Pick, C.G., Yanai, J., Alleva, E., 1992. Eight-arm maze performance, neophobia, and hippocampal cholinergic alterations after prenatal oxazepam in mice. Brain Res. Bull. 29, 609–616.

Lee, E.H., Tsai, M.J., 1989. The hippocampus and amygdala mediate the locomotor stimulating effects of corticotropin-releasing factor in mice. Behav. Neural Biol. 51, 412–423.

Lee, E.H., Tang, Y.P., Chai, C.Y., 1987. Stress and corticotropin-releasing factor potentiate center region activity of mice in an open field. Psychopharmacology (Berl.) 93, 320–323.

Liang, K.C., Lee, E.H., 1988. Intra-amygdala injections of corticotropin releasing factor facilitate inhibitory avoidance learning and reduce exploratory behavior in rats. Psychopharmacology (Berl.) 96, 232–236.

Lopez, F., Miller, L.G., Greenblatt, D.J., Paul, S.M., Shader, R.I., 1988. Low-dose alprazolam augments motor activity in mice. Pharmacol. Biochem. Behav. 30, 511–513.

Lopez, F., Miller, L.G., Greenblatt, D.J., Schatzki, A., Lumpkin, M., Shader, R.I., 1992. Chronic low-dose alprazolam augments gamma-aminobutyric acid(A) receptor function. J. Clin. Psychopharmacol. 12, 119–123.

Lucki, I., Ward, H.R., Frazer, A., 1989. Effect of 1-(*m*-chlorophenyl)piperazine and 1-(*m*-trifluoromethylphenyl)piperazine on locomotor activity. J. Pharmacol. Exp. Ther. 249, 155–164.

Mar, A., Spreekmeester, E., Rochford, J., 2000. Antidepressants preferentially enhance habituation to novelty in the olfactory bulbectomized rat. Psychopharmacologia 150, 52–60.

Marin, R.H., Martijena, I.D., Arce, A., 1997. Effect of diazepam and A beta-carboline on open-field and T-maze behaviors in 2-day-old chicks. Pharmacol. Biochem. Behav. 58, 915–921.

Matsubara, K., Matsushita, A., 1982. Changes in ambulatory activities and muscle relaxation in rats after repeated doses of diazepam. Psychopharmacology 77, 279–283.

Matto, V., Allikmets, L., 1999. Acute and chronic citalopram treatment differently modulates rat exploratory behavior in the exploration box test: no evidence for increased anxiety or changes in the [3H]raclopride binding. Pharmacology 58, 59–69.

McCarthy, M.M., Felzenberg, E., Robbins, A., Pfaff, D.W., Schwartz-Giblin, S., 1995. Infusions of diazepam and allopregnanolone into the midbrain central gray facilitate open-field behavior and sexual receptivity in female rats. Horm. Behav. 29, 279–295.

McNamara, R.K., Skelton, R.W., 1992. Like diazepam, CL 218,872, a selective ligand for the benzodiazepine omega 1 receptor subtype, impairs place learning in the Morris water maze. Psychopharmacology 107, 347–351.

Meert, T.F., 1992. Ritanserin overcomes exploratory inhibition induced by cocaine withdrawal. Behav. Pharmacol. 3, 149–154.

Meert, T.F., Colpaert, F.C., 1986. The shock probe conflict procedure. A new assay responsive to benzodiazepines, barbiturates and related compounds. Psychopharmacology (Berl.) 88, 445–450.

Meert, T.F., Melis, W., Aerts, N., Clincke, G., 1997. Antagonism of meta-chlorophenylpiperazine-induced inhibition of exploratory activity in an emergence procedure, the open field test, in rats. Behav. Pharmacol. 8, 353–363.

Meng, I.D., Drugan, R.C., 1993. Sex differences in open-field behavior in response to the beta-carboline FG 7142 in rats. Physiol. Behav. 54, 701–705.

Miller, L.G., Greenblatt, D.J., Roy, R.B., Gaver, A., Lopez, F., Shader, R.I., 1989a. Chronic benzodiazepine administration: III. Upregulation of gamma-aminobutyric acidA receptor binding and function associated with chronic benzodiazepine antagonist administration. J. Pharmacol. Exp. Ther. 248, 1096–1101.

Miller, L.G., Woolverton, S., Greenblatt, D.J., Lopez, F., Roy, R.B., Shader, R.I., 1989b. Chronic benzodiazepine administration: IV. Rapid development of tolerance and receptor downregulation associated with alprazolam administration. Biochem. Pharmacol. 38, 3773–3777.

Miller, L.G., Galpern, W.R., Greenblatt, D.J., Lumpkin, M., Shader, R.I., 1990. Chronic benzodiazepine administration: VI. A partial agonist produces behavioral effects without tolerance or receptor alterations. J. Pharmacol. Exp. Ther. 254, 33–38.

Misslin, R., Cigrang, M., 1986. Does neophobia necessarily imply fear or anxiety? Behav. Proc. 12, 45–50.

Moreau, J.L., Kilpatrick, G., Jenck, F., 1997. Urocortin, a novel neuropeptide with anxiogenic-like properties. NeuroReport 8, 1697–1701.

Moreira, E.G., Nascimento, N., Rosa, G.J., Vassilieff, V.S., 1996. Crotoxin-induced behavioral effects in rats. Braz. J. Med. Biol. Res. 29, 629–632.

Moreira, E.G., Nascimento, N., Rogero, J.R., Vassilieff, V.S., 2000. Gabaergic-benzodiazepine system is involved in the crotoxin-induced anxiogenic effect. Pharmacol. Biochem. Behav. 65, 7–13.

Moriarty, D.D., 1995. Anxiogenic effects of a beta-carboline on tonic immobility and open field behavior in chickens (*Gallus gallus*). Pharmacol. Biochem. Behav. 51, 795–798.

Nakamura-Palacios, E.M., De Oliveira, R.W., Gomes, C.F., 1999. Effects of diazepam or haloperidol on convulsion and behavioral responses induced by bilateral electrical stimulation in the medial prefrontal cortex. Prog. Neuropsychopharmacol. Biol. Psychiatry 23, 1369–1388.

Nazar, M., Jessa, M., Plaznik, A., 1997. Benzodiazepine–GABAA receptor complex ligands in two models of anxiety. J. Neural. Transm. 104, 733–746.

Nazar, M., Siemiatkowski, M., Czonkowska, A., Sienkiewicz-Jarosz, H., Plaznik, A., 1999a. The role of the hippocampus and 5-HT/GABA

interaction in the central effects of benzodiazepine receptor ligands. J. Neural. Transm. 106, 369–381.

Nazar, M., Siemiatkowski, M., Bidzinski, A., Czlonkowska, A., Sienkiewicz-Jarosz, H., Plaznik, A., 1999b. The influence of serotonin depletion on rat behavior in the Vogel test and brain 3H-zolpidem binding. J. Neural Transm. 106, 355–368.

Nobrega, J.N., Coscina, D.V., 1983. Central injections of the GABA-transaminase inhibitor ethanolamine-*O*-sulfate (EOS): effects on brain [14C]2-deoxy-D-glucose uptake and behavior in rats. Brain Res. 262, 243–252.

Novas, M.L., Wolfman, C., Medina, J.H., De Robertis, E., 1988. Proconvulsant and 'anxiogenic' effects of *n*-butyl beta carboline-3-carboxylate, an endogenous benzodiazepine binding inhibitor from brain. Pharmacol. Biochem. Behav. 30, 331–336.

O'Connor, W.T., Earley, B., Leonard, B.E., 1985. Antidepressant properties of the triazolobenzodiazepines alprazolam and adinazolam: studies on the olfactory bulbectomized rat model of depression. Br. J. Clin. Pharmacol. 19 (Suppl. 1), 49S–56S.

Pähkla, R., Harro, J., Rago, L., 1996. Behavioural effects of pinoline in the rat forced swimming, open field and elevated plus-maze tests. Pharmacol. Res. 34, 73–78.

Panickar, K.S., McNaughton, N., 1991. Comparison of the effects of buspirone and chlordiazepoxide on successive discrimination. Pharmacol. Biochem. Behav. 39 (2), 275–278 (June).

Papp, M., Przegalinski, E., 1989. The 5-HT3 receptor antagonists ICS 205-930 and GR38032F, putative anxiolytic drugs, differ from diazepam in their pharmacological profile. J. Psychopharmacol. 3, 14–20.

Paré, A.M.T., Paré, W.P., Kluczynski, J., 1999. Negative affect and voluntary alcohol consumption in Wistar-Kyoto (WKY) and Sprague-Dawley Rats. Physiol. Behav. 67 (2), 219–225 (15 August).

Plaznik, A., Stefanski, R., Palejko, W., Kostowski, W., 1991. Serotonergic mechanisms in the nucleus accumbens affected by chronic desipramine treatment. Pharmacol. Biochem. Behav. 39, 43–48.

Podhorna, J., Franklin, K.B., 2000. Pontine tegmentum lesions increase anxiety-like behavior in rats: a comparison with anxiety produced by beta-CCE. Pharmacol. Biochem. Behav. 65, 267–273.

Poggioli, R., Vergoni, A.V., Rasori, E., Marrama, D., Bertolini, A., 1992. Behavioral effects of atriopeptin in rats. Neuropeptides 22, 149–154.

Pohorecky, L.A., Roberts, P., 1991. Activity in a modified open-field apparatus: effect of diazepam and prenatal stress. Neurotoxicol. Teratol. 13, 129–133.

Pritchard, G.A., Galpern, W.R., Lumpkin, M., Miller, L.G., 1991. Chronic benzodiazepine administration: VIII. Receptor upregulation produced by chronic exposure to the inverse agonist FG-7142. J. Pharmacol. Exp. Ther. 258, 280–285.

Ramanathan, M., Jaiswal, A.K., Bhattacharya, S.K., 1998. Differential effects of diazepam on anxiety in streptozotocin induced diabetic and non-diabetic rats. Psychopharmacology (Berl.) 135, 361–367.

Ramboz, S., Oosting, R., Amara, D.A., Kung, H.F., Blier, P., Mendelsohn, M., Mann, J.J., Brunner, D., Hen, R., 1998. Serotonin receptor 1A knockout: an animal model of anxiety-related disorder. Proc. Natl. Acad. Sci. U. S. A. 95, 4476–4481.

Reddy, D.S., Kaur, G., Kulkarni, S.K., 1998. Sigma (sigma1) receptor mediated anti-depressant-like effects of neurosteroids in the Porsolt forced swim test. NeuroReport 9, 3069–3073.

Rex, A., Stephens, D.N., Fink, H., 1996. "Anxiolytic" action of diazepam and abecarnil in a modified open field test. Pharmacol. Biochem. Behav. 53, 1005–1111.

Rex, A., Voigt, J.P., Voits, M., Fink, H., 1998. Pharmacological evaluation of a modified open-field test sensitive to anxiolytic drugs. Pharmacol. Biochem. Behav. 59, 677–683.

Rizzi, C.A., Prudentino, A., Giraldo, E., 1993. Effects on general behaviour and neurotransmitter functions of a new 5-hydroxytryptamine3 receptor antagonist with potential therapeutic relevance in central nervous system disturbances. Arzneimittelforschung 43, 1033–1041.

Salvatierra, N.A., Arce, A., 2001. Day-old chicks categorised on latency to peck, exhibit a stable fear pattern until 15 days of age. Appl. Anim. Behav. Sci. 73, 103–116.

Sanger, D.J., Zivkovic, B., 1988. Further behavioural evidence for the selective sedative action of zolpidem. Neuropharmacology 27, 1125–1130.

Schmitt, U., Hiemke, C., 1998. Combination of open field and elevated plus-maze: a suitable test battery to assess strain as well as treatment differences in rat behavior. Prog. Neuropsychopharmacol. Biol. Psychiatry 22, 1197–1215.

Schmitt, U., Hiemke, C., 1999. Effects of GABA-transporter (GAT) inhibitors on rat behaviour in open-field and elevated plus-maze. Behav. Pharmacol. 10, 131–137.

Schmitt, U., Luddens, H., Hiemke, C., 2000. Behavioral effects of GABA(A) receptor stimulation and GABA-transporter inhibition. Pharmacol. Biochem. Behav. 65, 351–356.

Schmitt, U., Luddens, H., Hiemke, C., 2001. Behavioral analysis indicates benzodiazepine-tolerance mediated by the benzodiazepine binding-site at the GABA(A)-receptor. Prog. Neuropsychopharmacol. Biol. Psychiatry 25, 1145–1160.

Semeniuk, T., Jhangri, G.S., Le Melledo, J.M., 2001. Neuroactive steroid levels in patients with generalized anxiety disorder. J. Neuropsychiatry Clin. Neurosci. 13, 396–398.

Seredenin, S.B., Blednov Yu, A., Badyshtov, B.A., Gordey, M.L., Nagovitsina, Y.A., 1990. Pharmacogenetic analysis of mechanisms of emotional stress: effects of benzodiazepines. Ann. Ist. Super. Sanita 26, 81–87.

Sherif, F., Oreland, L., 1994. Effects of chronic treatment with the GABA-transaminase inhibitor vigabatrin on exploratory behaviour in rats. Behav. Brain Res. 63, 11–15.

Sherif, F., Oreland, L., 1995. Effect of the GABA-transaminase inhibitor vigabatrin on exploratory behaviour in socially isolated rats. Behav. Brain Res. 72, 135–140.

Siemiatkowski, M., Sienkiewicz-Jarosz, H., Czlonkowska, A.I., Bidzinski, A., Plaznik, A., 2000. Effects of buspirone, diazepam, and zolpidem on open field behavior, and brain [3H]muscimol binding after buspirone pretreatment. Pharmacol. Biochem. Behav. 66, 645–651.

Silva, M.R., Felicio, L.F., Nasello, A.G., Bernardi, M.M., 1995. Is perinatal picrotoxin anxiogenic? Braz. J. Med. Biol. Res. 28, 663–666.

Singh, Y., Jaiswal, A.K., Singh, M., Bhattacharya, S.K., 1996. Behavioural effects of prenatal diazepam administration on anxiety patterns in rats. Indian J. Exp. Biol. 34, 1095–1099.

Skutella, T., Probst, J.C., Renner, U., Holsboer, F., Behl, C., 1998. Corticotropin-releasing hormone receptor (type I) antisense targeting reduces anxiety. Neuroscience 85, 795–805.

Stefanski, R., Palejko, W., Kostowski, W., Plaznik, A., 1992. The comparison of benzodiazepine derivatives and serotonergic agonists and antagonists in two animal models of anxiety. Neuropharmacology 31, 1251–1258.

Stefanski, R., Palejko, W., Bidzinski, A., Kostowski, W., Plaznik, A., 1993. Serotonergic innervation of the hippocampus and nucleus accumbens septi and the anxiolytic-like action of midazolam and 5-HT1A receptor agonists. Neuropharmacology 32, 977–985.

Stone, E.A., Manavalan, S.J., Yi, Z., Quartermain, D., 1995. Beta adrenoceptor blockade mimics effects of stress on motor activity in mice. Neuropsychopharmacology 12 (1), 65–71 (February).

Stratton, S.C., Beresford, I.J.M., Elliot, P.J., Hagan, R.M., 1993. Behavioural effects of centrally infused tachykinin NK2 receptor agonists and antagonists in rat models of anxiety. J. Psychopharmacol. 7, A11. (Suppl.).

Sutton, R.E., Koob, G.F., Le Moal, M., Rivier, J., Vale, W., 1982. Corticotropin releasing factor produces behavioural activation in rats. Nature 297, 331–333.

Taira, T., Porkka-Heiskanen, T., Korpi, E.R., 1992. Neonatal administration of a GABA-T inhibitor alters central GABAA receptor mechanisms and alcohol drinking in adult rats. Psychopharmacology 109, 191–197.

Taira, T., Uusi-Oukari, M., Korpi, E.R., 1993. Early postnatal treatment

with muscimol transiently alters brain GABAA receptors and open-field behavior in rat. Eur. J. Pharmacol. 230, 307–312.

Takahashi, L.K., Kalin, N.H., 1989. Role of corticotropin-releasing factor in mediating the expression of defensive behavior. In: Blanchard, R.J., Brain, P.F., Blanchard, D.C., Parmigiani, S. (Eds.), Ethoexperimental Approaches to the Study of Behavior. NATO ASI Series. Kluwer Academic Publishing, Kluwer, Dordrecht, Boston, London, pp. 580–594.

Tashma, Z., Raveh, L., Liani, H., Alkalay, D., Givoni, S., Kapon, J., Cohen, G., Alcalay, M., Grauer, E., 2001. Bretazenil, a benzodiazepine receptor partial agonist, as an adjunct in the prophylactic treatment of OP poisoning. J. Appl. Toxicol. 21 (Suppl. 1), S115–S119.

Thomsen, P.H., 2000. Obsessive-complusive disorder: pharmacological treatment. Eur. Child Adolesc. Psychiatry 9, I76–I84 (Suppl.).

Ueki, S., 1987. Behavioral pharmacology of zopiclone. Sleep 10 (Suppl. 1), 1–6.

Ueki, S., Watanabe, S., Yamamoto, T., Shibata, S., Shibata, K., 1984. Behavioral effects of brotizolam, a new thienotriazolodiazepine derivative. Jpn. J. Pharmacol. 35, 287–299.

Ueki, S., Watanabe, S., Fujiwara, M., Yamamoto, T., Shibata, S., Shibata, K., Ohta, H., Sakurai, Y., Iwasaki, K., Liou, S.Y., et al., 1985. Behavioral and electroencephalographic effects of lormetazepam. Nippon Yakurigaku Zasshi 86, 145–163.

Uhlenhuth, E.H., Matuzas, W., Glass, R.M., Easton, C., 1989. Response of panic disorder to fixed doses of alprazolam or imipramine. J. Affect. Disord. 17, 261–270.

Wagstaff, A.J., Cheer, S.M., Matheson, A.J., Ormrod, D., Goa, K.L., 2002a. Paroxetine: an update of its use in psychiatric disorders in adults. Drugs 62, 655–703.

Wagstaff, A.J., Cheer, S.M., Matheson, A.J., Ormrod, D., Goa, K.L., 2002b. Spotlight on paroxetine in psychiatric disorders in adults. CNS Drugs 16, 425–434.

Walsh, R.N., Cummins, R.A., 1976. The open field test: a critical review. Psychol. Bull. 83, 481–504.

Westenberg, H.G., 1996. Developments in the drug treatment of panic disorder: what is the place of the selective serotonin reuptake inhibitors? J. Affect. Disord. 40, 85–93.

Wikinski, S.I., Acosta, G.B., Gravielle, M.C., Bonavita, C.D., Bisagno, V., Fiszer de Plazas, S., Rubio, M.C., 2001. Diazepam fails to potentiate GABA-induced chloride uptake and to produce anxiolytic-like action in aged rats. Pharmacol. Biochem. Behav. 68, 721–727.

Williams, J.H., Meara, J.R., Azmitia, E.C., 1990. Effects of 5,7-dihydroxytryptamine injections in the fornix–fimbria on locomotor activity in photocell cages and the open field. Behav. Brain Res. 40, 37–44.

Wong, P.T., Ong, Y.P., 2001. Acute antidepressant-like and antianxiety-like effects of tryptophan in mice. Pharmacology 62, 151–156.

Wu, H.C., Chen, K.Y., Lee, W.Y., Lee, E.H., 1997. Antisense oligonucleotides to corticotropin-releasing factor impair memory retention and increase exploration in rats. Neuroscience 78, 147–153.

Yerbury, R.E., Cooper, S.J., 1987. The benzodiazepine partial agonists, Ro16-6028 and Ro17-1812, increase palatable food consumption in nondeprived rats. Pharmacol. Biochem. Behav. 28, 427–431.

Zorrilla, E.P., Schulteis, G., Ling, N., Koob, G.F., De Souza, E.B., 1998. Performance-enhancing ffects of corticotropin-releasing factor binding protein ligand inhibitors. Soc. Neurosci. Abstr. 24, 234.6.

Pharmacology, Biochemistry and Behavior 90 (2008) 748–752

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

The neurotensin-1 receptor agonist PD149163 blocks fear-potentiated startle

Paul D. Shilling, David Feifel *

Department of Psychiatry, University of California, San Diego, United States

ARTICLE INFO

Article history:
Received 12 December 2007
Received in revised form 29 May 2008
Accepted 30 May 2008
Available online 7 June 2008

Keywords:
Neurotensin
Anxiety
Fear-potentiated
Startle
Anxiolytic
Animal model

ABSTRACT

Preliminary evidence suggests that the neuropeptide, neurotensin (NT) may regulate fear/anxiety circuits. We investigated the effects of PD149163, a NT1 receptor agonist, on fear-potentiated startle (FPS). Sprague Dawley rats were trained to associate a white light with a mild foot shock. In one experiment, animals were treated with either subcutaneous vehicle or PD149163 (0.01, 0.1 or 1.0 mg/kg) 24 h after training. Twenty minutes later their acoustic startle response in the presence or absence of the white light was tested. In a second experiment, saline and 1.0 mg/kg PD149163 were tested using a separate group of rats. In the first experiment, PD149163 produced a non-significant decrease in baseline acoustic startle at all three doses. As expected, saline-treated rats exhibited significant FPS. An ANOVA of percentage FPS revealed no significant effect of treatment group overall but the high dose group did not display FPS strongly suggesting an FPS effect at this dose. This finding was confirmed in the second experiment where the high dose of PD149163 reduced percent FPS relative to saline ($P<0.05$). These data suggest that systemically administered NT1 agonists modulate the neural circuitry that regulates fear and anxiety to produce dose-dependent anxiolytic-like effects on FPS.

1. Introduction

Neurotensin (NT) is a tridecapeptide with a wide distribution throughout the mammalian central nervous system (Emson et al., 1982). When administered directly into the brain, NT has been reported to have several antipsychotic-like behavioral and neurochemical effects including inhibition of mesolimbic dopamine function, antagonism of stimulant-induced hyperlocomotion (Kalivas et al., 1984; Robledo et al., 1993; Skoog et al., 1986) and stimulant-induced disruption of sensorimotor gating (Feifel et al., 1997). NT does not effectively reach the brain after systemic administration, nor does the C-terminal hexapeptide, NT(8–13), the smallest NT peptide fragment which contains full biological activity of the parent peptide (Kanba et al., 1988; Machida et al., 1993).

In order to produce viable drug candidates, several NT mimetics have been produced by chemically modifying the NT(8–13) peptide to make it more resistant to endopeptidase degradation in the periphery and thus better able to enter the central nervous system (CNS) (Cusack et al., 2000; Wustrow et al., 1995). PD149163 is one such NT mimetic produced by adding a reduced amide bond to NT(8–13) (Wustrow et al., 1995). PD149163 has strong and selective affinity for the neurotensin-1 (NT1) receptor (Petrie et al., 2004), the NT receptor type implicated in the antipsychotic-like effects of NT. PD149163 has been shown to produce robust antipsychotic-like effects (Feifel et al., 1999). Recently, PD149163 has been shown to produce pro-cognitive effects in the CNS after systemic administration (Azrni et al., 2006).

There is strong evidence that NT1 agonists modulate mesolimbic dopamine and forebrain acetylcholine transmission in the brain and this effect has been presumed to underlie the antipsychotic-like and pro-cognitive effects, respectively, of NT and NT mimetics such as PD149163 (Nakachi et al., 1995; Szigethy and Beaudet, 1987). Our laboratory discovered that NT mimetics have more diverse pharmacological effects, which raises the possibility that they may have important actions on other circuits relevant to neuropsychiatric disorders. For example, we reported that systemic administration of PD149163 blocks the behavioral effects of a serotonin-2 (5-HT2) agonist, DOI, suggesting that NT agonists also have inhibitory effects on serotoninergic transmission at 5-HT2 receptors (Feifel et al., 2003b). Serotoninergic mechanisms have been strongly implicated in anxiety and depression, and inhibition of 5-HT2 receptors specifically may be a mechanism for anxiolysis and anti-depression (Mora et al., 1997; Weisstaub et al., 2006). Other evidence also suggests that NT agonists may regulate anxiety-relevant neurocircuitry. NT is localized in several brain regions that have been associated with fear and anxiety, such as the amygdala and hippocampus (Campeau et al., 1992; Davis et al., 1993; Gewirtz et al., 2000; Paxinos and Watson 1997). Saiz Ruiz et al. (1992) reported that NT levels were significantly decreased in patients with anxiety and were normalized after recovery. In a fear conditioning test, duration of freezing was significantly reduced by beta lactotensin, a natural ligand for NT receptors (Yamauchi et al., 2006). In addition, Shugalev et al. (2005) found that NT injections into the substantia nigra reduced fear produced by serotoninergic lesions of the dorsal raphe (Shugalev et al., 2005). As

* Corresponding author. Department of Psychiatry, UCSD, 200 West Arbor Drive, San Diego, CA 92103-8218. United States.
E-mail address: dfeifel@ucsd.edu (D. Feifel).

0091-3057/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2008.05.025

these studies did not use NT receptor selective agonists, the role of the NT1 receptor in these anti-fear effects is not known. In order to investigate the role of NT1 receptors in fear circuits and to further investigate the anxiolytic potential of NT1 agonists, we tested the effects of PD149163 on fear-potentiated startle (FPS), a commonly employed animal model of anticipatory anxiety.

FPS is decreased by drugs that reduce fear and anxiety in humans such as benzodiazepines, whereas, it is increased by drugs that have anxiogenic effects, such as yohimbine (Davis et al., 1993). Therefore FPS has been used extensively to test the anxiolytic potential of many compounds (Davis et al., 1993; Walker and Davis 2002). In this paradigm, the magnitude of the acoustic startle reflex can be enhanced by a conditioned stimulus such as a light that has previously been associated with a shock. Fear-potentiated startle is exhibited when the startle reflex is significantly greater when the conditioned stimulus is present.

2. Methods

2.1. Animals

Seventy-six male Sprague Dawley rats (275–375 g at testing) were obtained from Harlan Laboratories, San Diego, California. Animals were housed in groups of two in clear plastic chambers in a climate-controlled room on a 12:12 hour light/dark cycle (lights on 7:00 am–7:00 pm). The rats were handled prior to testing. All testing occurred during the light phase of the rats' circadian illumination schedule and they were allowed free access to food and water for the extent of the study, except during the actual testing. Behavioral testing was performed between 9:00 am and 4:30 pm beginning a minimum of 7 days after arrival. All studies described in this publication were carried out in accordance with the "Principles of laboratory and animal care" as described in the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health (Publication No. 85-23, revised 1985).

2.2. Drugs

PD149163 was generously made available by the NIMH Chemical Synthesis and Drug Supply Program (Washington D.C.), and SRI International (Menlo Park, California).

2.3. Startle testing and test sessions

Startle testing was performed in two identical startle chambers obtained from San Diego Instruments (San Diego, California). Each chamber consisted of a clear non-restrictive Plexiglas cylinder resting on a Plexiglas platform inside a ventilated enclosure, housed in a sound-attenuated room. A continuous background noise of 65 dB, as well as the various acoustic stimuli, were produced within each chamber by a high-frequency loudspeaker (Radio Shack Supertweeter, San Diego, CA). The whole-body startle response of each animal produced vibrations of the Plexiglas cylinder, which were transduced into analog signals by a piezoelectric unit, mounted underneath the Plexiglas platform (Mansbach et al., 1988). These analog signals were then digitized and stored by an interface unit connected to a microcomputer. Startle amplitude was defined as the amount of motion detected by the piezoelectric unit. The startle stimulus was a 40 ms 95 dB burst of white noise.

2.4. Baseline matching

Approximately three days before fear conditioning rats were placed in startle chambers for 5 min prior to exposure to 30 95 dB startle stimuli in the dark. This session was used to match groups of animals for similar levels of startle.

2.5. Training

For two consecutive days animals were placed in dark startle chambers for 5 min prior to exposure to 10 light–shock pairings. Each light–shock pairing consisted of a 3.7 s light (15 W, 6 in. from chambers) and a 0.6 mA shock delivered during the last 0.5 s of light exposure. The shock was generated by shockers connected to a grid (San Diego Instruments) covering the bottom of the startle chamber. The average intertrial interval was 2 min (ranging from 1–3 min).

2.6. Potentiated startle testing

On the day following startle training animals were injected with either saline, or PD149163 (0.01, 0.1 or 1.0 mg/kg). Twenty minutes later they were placed in dark startle chambers and exposed to 5 min of background noise followed by 10 95 dB stimuli. Rats were then presented with 20 95 dB trials (noise-only) in the dark and 20 trials where the 95 dB noise bursts were presented 3.2 s after the onset of the light (light+noise). Interstimulus intervals were all 30 s. Rats were randomly assigned to receive one of two types of startle sessions. In one session type, 20 noise-only stimuli were presented followed by 20 light+noise stimuli. In the other session type, the order of trial presentation was reversed so that 20 light+noise stimuli were presented initially, followed by 20 noise-only presentations, in order to control for any order effect of the stimulus-type. We used this paradigm rather than using one session type with randomly presented noise–startle and startle-alone trials throughout the session because in parametric studies that we conducted prior to this experiment, this paradigm (non-random assignment) produced more robust FPS than random presentation. We hypothesize this is because the CS produces an emotional arousal (fear) in animals that likely lasts longer than the very short 30 second interval between the startle stimuli. Therefore, the arousal produced by CS likely "leaks" over to startle-alone presentations that may follow it. By separating CS–startle and startle-alone presentation into different halves of the startle session, the carry-over phenomenon is likely mitigated. Counterbalancing the order of presentation as we did controls for any order effect.

Two separate experiments were performed. The first experiment was a dose–response study in which thirty-six rats were administered SC injections of either saline or PD149163 (0.01, 0.1 or 1.0 mg/kg). After reviewing the results of the first experiment, we performed a second experiment to confirm an apparent effect on FPS observed with the highest dose in the first experiment. In this second experiment a new set of forty rats were treated with either saline or 1.0 mg/kg PD149163 and tested in the same FPS paradigm.

2.7. Data analysis

The first ten 95 dB stimuli of the startle session were used to habituate animals to the 95 dB noise bursts and were not used in the data analysis. Data were reviewed and any animal that exhibited startle values less than 10 or greater than 3.0 standard deviations (SD) from the group mean (outlier) were excluded from the data analysis. In the dose–response study, one animal in the group receiving the middle dose of PD149163 and one in the high dose exhibited startle values that were less than 10 and were not included in the data analysis. One animal in the low dose group of that study exhibited startle that was greater that 3 SD from the mean and was excluded. Eight PD149163-treated animals that exhibited startle values less than 10 in the second study were excluded from data analysis. There were 16 rats in each group after eliminating the "non-startlers" in experiment #2.

A percent score for FPS was calculated for each animal by using the following formula: [(mean startle magnitude on light-noise trials − mean startle magnitude on noise alone trials)/mean startle magnitude on noise alone trials]×100 (Walker and Davis, 2002; Winslow, Noble and Davis, 2007). We analyzed these data using percent FPS vs. absolute

change in startle levels ((startle + light) − startle-alone) to compensate for between group and individual differences in startle levels. Calculating FPS in each rat as a percentage increase of their own baseline startle, controls for differences in baseline startle among the rats, whether due to inherent genetic differences or differences produced by the different drug treatments (e.g., PD149163 doses versus saline). This is a common approach used, for example in studies of prepulse inhibition of startle, another modified startle measure, where percent PPI is typically measured to control for potential confounding effects of drugs on baseline startle. Paired *t*-tests were used to determine if the conditioned stimulus (light) significantly increased startle magnitude (i.e., produced FPS).

In the dose–response study, the effects of the PD149163 startle response (noise alone) on FPS were analyzed using a one-way ANOVA with drug treatment as a between subjects factor. Planned group-wise *t*-tests, corrected for multiple comparisons using the Bonferonni method, were also used to compare each PD149163 dose group with the saline-treated group. In the second experiment, in which only one saline and one dose of PD149163 was tested, an independent *t*-test were used to compare the startle response in saline-treated versus the PD149163-treated animals and the same approach was used for FPS data. Startle (95 dB stimuli presented after the first 10 trials) data were analyzed in a similar manner to the FPS data.

3. Results

3.1. Baseline startle

In the first experiment, PD149163 produced a non-significant trend towards a reduction in startle magnitude at all three doses, $F(3, 27) = 1.70$, NS (Fig. 1A). In the second experiment, PD149163 significantly decreased startle magnitude ($t(30) = 2.04$, $P < 0.01$) (Fig. 2A). Startle magnitude values in saline and PD149163 treated animals were comparable to those in the first experiment.

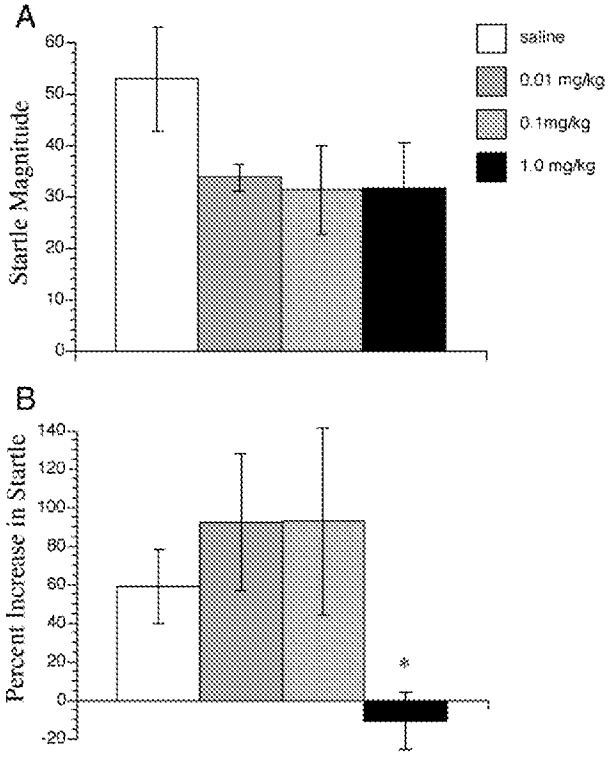

Fig. 1. The effects of PD149163 on startle magnitude (A) and fear-potentiated startle (B). Fear-potentiated startle data are represented as percent scores [(mean startle magnitude on light-noise trials − mean startle magnitude on noise alone trials)/mean startle magnitude on noise alone trials] × 100. PD149163 significantly different from saline represented by * $P < 0.05$. Data points represent the mean + SEM ($n = 7–10$).

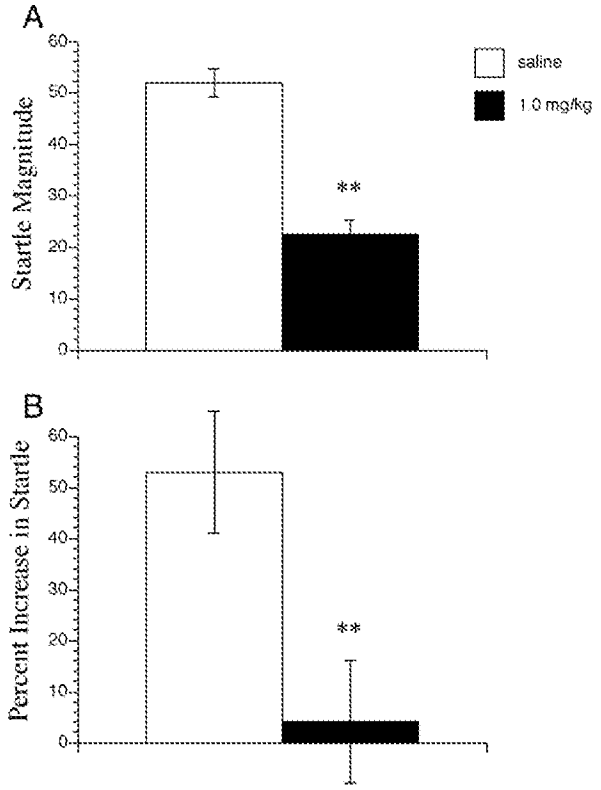

Fig. 2. The effects of PD149163 on startle magnitude (A) and fear-potentiated startle (B). Fear-potentiated startle data are represented as percent scores [(mean startle magnitude on light-noise trials − mean startle magnitude on noise alone trials)/mean startle magnitude on noise alone trials] × 100. PD149163 significantly different from saline represented by ** $P < 0.01$. Data points represent the mean + SEM ($n = 16$).

3.2. Fear-potentiated startle

Comparison of the startle response to noise alone versus light plus noise in saline-treated rats revealed that significant fear-potentiation of startle was exhibited in both the first, dose–response experiment ($t(10) = 1.83$, $P < 0.05$), and the second experiment ($t(15) = 1.75$, $P < 0.01$). Startle was potentiated on average by 60% and 53%, respectively in the saline-treated groups of the two experiments (Figs. 1B and 2B).

In the dose–response study, ANOVA revealed no significant main effect of drug treatment on percent FPS ($F(3,27) = 2.30$, $P = 0.100$. However, visual inspection and the percent FPS data revealed that the low and middle doses of PD149163 produced a modest non-significant increase in FPS, whereas FPS was completely blocked at the highest dose of PD149163. Planned post-hoc *t*-tests revealed that FPS at the highest dose, but not the other two doses of PD149163 differed significantly from the FPS in the saline-treated group ($t(15) = 0.502$, $P < 0.05$).

In the second experiment, which was performed to corroborate the apparent effect of high dose PD149163 on FPS, animals treated with the highest dose of PD149163 exhibited significantly decreased percent FPS compared to those treated with saline, ($t(30) = 1.70$, $P < 0.01$) (Fig. 2B).

4. Discussion

Light as a conditioned fear stimulus produced a significant enhancement in the acoustic startle response demonstrating that FPS was successfully induced by the training procedures in these experiments. The findings indicate that the highest dose of systemically administered PD149163 blocked FPS in Sprague Dawley rats. Although the ANOVA used to analyze the FPS data in the first experiment did not produce a significant main effect of overall drug treatment, this was likely due to the

large variance in some of the groups (i.e., low and middle dose of PD149163). In addition, individual comparisons of each PD149163 dose to saline in that experiment revealed a significant difference in the highest PD149163 dose compared to saline. This result was reproduced in the second experiment using only the highest dose and saline in different rats.

In addition to blocking FPS, PD149163 produced a reduction of startle magnitude at all three doses. It is conceivable that this reduction in baseline startle could have affected our FPS results. However, it is unlikely that the attenuation of FPS was an artifact of the decrease in startle produced by this NT1 agonist. In this respect, a lowering of the baseline startle is more likely to produce secondary enhancement of FPS rather than reduction in FPS, as it would be easier to potentiate low baseline startle than high baseline startle. Furthermore, the dose–response pattern revealed in the dose–response study provides a compelling argument against reduction in FPS as secondary to the suppression of baseline startle. All three doses of PD149163 decreased startle magnitude to a similar degree but there were distinct dose–effects on FPS. The low and middle doses produced a non-significant enhancement of FPS while the highest dose inhibited FPS. This result demonstrates a dissociation in the effects of PD149163 on startle and FPS in this experiment and that it is unlikely that inhibition of FPS by PD149163 is an artifact of the decreased startle magnitude produced by this compound. This pattern of drug effects on baseline startle and FPS has been reported with other drugs that are known anxiolytics such as diazepam (Berg and Davis, 1984). In contrast, buspirone, another known anxiolytic, increases baseline startle but decreases FPS (Kehne et al., 1988; Mansbach and Geyer, 1988), providing further evidence that drug effects on baseline startle and FPS can be dissociated. Nevertheless to the extent that reduction in baseline startle is proposed as predictive of an anxiolytic, it is noteworthy that PD149163 reduced both baseline startle and FPS.

It is not totally clear where in the brain PD149163 acts to block FPS. However, a strong possibility would be the amygdala, a region implicated in a variety of anxiety disorders (Britton et al., 2005; Cannistraro et al., 2004; Milham et al., 2005). The central amygdala (CEA) is necessary for the acquisition and expression of conditioned fear via a visual cue (Davis and Shi, 2000; LeDoux, 2000). NT is co-expressed in GABAergic neurons in the CEA (Batten et al., 2002) and NT1 receptors are also localized in this brain region (Alexander and Leeman, 1998). Centrally administered NT increases c-*fos* and zif268 expression in the CEA (Lambert et al., 1996) suggesting that NT activates this brain region. However, Beck and Fibiger (1995) reported that drug-induced c-*fos* levels in the CEA may not be associated with the attenuation of FPS (Beck and Fibiger, 1995). Further work will be needed to determine if the CEA mediates NT-induced blockade of FPS.

NT1 agonists have previously been reported to exhibit antipsychotic-like, anti-addiction and pro-cognitive effects in animal models (Azmi et al., 2006; Boules et al., 2005, 2007; Feifel et al., 2007, 2003a,b, 2004, 1999; Fredrickson et al., 2005; Richelson et al., 2005; Shilling et al., 2004, 2003). These data suggest that NT1 agonists such as PD149163 may also modulate anxiety-related circuits and the NT1 receptor system may be a novel target for anxiolytic drugs. Given that PD149163 has been shown to increase memory (Azmi et al., 2006), it would be expected to increase memory of CS and therefore, increase FPS. Decreasing FPS indicates that it has a robust mitigating effect on FPS that can overcome the expected increase in FPS due to enhanced CS recall. To our knowledge only one other neuropeptide or neuropeptide-mimetic, has been shown to block FPS. In this regard, Myers et al. (2004) reported that secretin, a neurohormone neuropeptide, reduced FPS after systemic administration.

Benzodiazepines, and antidepressant drugs, particularly SSRI antidepressants, are the most widely used anti-anxiety agents with proven clinical efficacy (Rickels and Rynn 2002). Benzodiazepines generally reduce FPS after a single dose (Davis et al., 1993) whereas SSRIs tend not to (Burghardt et al., 2004). These effects are consistent with clinical experience that benzodiazepines produce anxiolytic effects in patients with anxiety disorders after a single injection, whereas SSRIs more typically require long-term use before producing anxiolytic effect (Feighner and Boyer, 1992; Goldstein and Goodnick, 1998; Goodnick and Goldstein, 1998). In this respect, it is interesting that a single administration of PD149163 was sufficient to reduce FPS, a time course more similar to benzodiazepines than SSRIs.

In summary, we have provided evidence that a selective NT1 receptor agonist, PD149163, can block the expression of fear-potentiated startle suggesting that the NT1 receptor is involved in modulation of fear circuits by NT and raising the possibility that NT1 agonists may be useful for the treatment of anxiety. Additional research testing other NT1 agonists and using other animal models of anxiety (e.g., elevated plus maze) are needed to substantiate this possibility.

Acknowledgements

DF and PDS are partially funded by NIMH grant (MH62451). We thank Gilia Melendez for her excellent technical assistance.

References

Alexander MJ, Leeman SE. Widespread expression in adult rat forebrain of mRNA encoding high-affinity neurotensin receptor. J Comp Neurol 1998;402:475–500.
Azmi N, Norman C, Spicer CH, Bennett GW. Effects of a neurotensin analogue (PD149163) and antagonist (SR142948A) on the scopolamine-induced deficits in a novel object discrimination task. Behav Pharmacol 2006;17:357–62.
Batten TF, Gamboa-Esteves FO, Saha S. Evidence for peptide co-transmission in retrograde- and anterograde-labelled central nucleus of amygdala neurones projecting to NTS. Auton Neurosci 2002;98:28–32.
Beck CH, Fibiger HC. Conditioned fear-induced changes in behavior and in the expression of the immediate early gene cfos: with and without diazapam. J. Neurosci. 1995;15 (1 Pt2):709–20.
Berg WK, Davis M. Diazepam blocks fear-enhanced startle elicited electrically from the brainstem. Physiol Behav 1984;32(2):333–6.
Boules M, Fredrickson P, Richelson E. Neurotensin agonists as an alternative to antipsychotics. Expert Opin Investig Drugs 2005;14:359–69.
Boules M, Shaw A, Fredrickson P, Richelson E. Neurotensin agonists: potential in the treatment of schizophrenia. CNS Drugs 2007;21:13–23.
Britton JC, Phan KL, Taylor SF, Fig LM, Liberzon I. Corticolimbic blood flow in posttraumatic stress disorder during script-driven imagery. Biol Psychiatry 2005;57:832–40.
Burghardt NS, Sullivan GM, McEwen BS, Gorman JM, LeDoux JE. The selective serotonin reuptake inhibitor citalopram increases fear after acute treatment but reduces fear with chronic treatment: a comparison with tianeptine. Biol Psychiatry 2004;55:1171–8.
Campeau S, Miserendino MJ, Davis M. Intra-amygdala infusion of the *N*-methyl-D-aspartate receptor antagonist AP5 blocks acquisition but not expression of fear-potentiated startle to an auditory conditioned stimulus. Behav Neurosci 1992;106:569–74.
Cannistraro PA, Wright CI, Wedig MM, et al. Amygdala responses to human faces in obsessive–compulsive disorder. Biol Psychiatry 2004;56:916–20.
Cusack B, Boules M, Tyler BM, Fauq A, McCormick DJ, Richelson E. Effects of a novel neurotensin peptide analog given extracranially on CNS behaviors mediated by apomorphine and haloperidol. Brain Res 2000;856:48–54.
Davis M, Shi C. The amygdala. Curr Biol 2000;10:R131.
Davis M, Falls WA, Campeau S, Kim M. Fear-potentiated startle: a neural and pharmacological analysis. Behav Brain Res 1993;58:175–98.
Emson PC, Goedert M, Horsfield P, Rioux F, St Pierre S. The regional distribution and chromatographic characterisation of neurotensin-like immunoreactivity in the rat central nervous system. J Neurochem 1982;38:992–9.
Feifel D, Minor KL, Dulawa S, Swerdlow NR. The effects of intra-accumbens neurotensin on sensorimotor gating. Brain Res 1997;760:80–4.
Feifel D, Reza TL, Wustrow DJ, Davis MD. Novel antipsychotic-like effects on prepulse inhibition of startle produced by a neurotensin agonist. J Pharmacol Exp Ther 1999;288:710–3.
Feifel D, Melendez G, Shilling PD. PD149163, a neurotensin agonist, attenuates prepulse inhibition deficits produced by dizocilpine and DOI. Neuropsychopharmacology 2003a;28:651–3.
Feifel D, Melendez G, Shilling PD. A systemically administered neurotensin agonist blocks disruption of prepulse inhibition produced by a serotonin-2A agonist. Neuropsychopharmacology 2003b;28:651–3.
Feifel D, Melendez G, Shilling PD. Reversal of sensorimotor gating deficits in Brattleboro rats by acute administration of clozapine and a neurotensin agonist, but not haloperidol: a potential predictive model for novel antipsychotic effects. Neuropsychopharmacology 2004;29:731–8.
Feifel D, Melendez G, Priebe K, Shilling PD. The effects of chronic administration of established and putative antipsychotics on natural prepulse inhibition deficits in Brattleboro rats. Behav Brain Res 2007;81:278–86.
Feighner JP, Boyer WF. Paroxetine in the treatment of depression: a comparison with imipramine and placebo. J Clin Psychiatry 1992;53:44–7 Suppl.

Fredrickson P, Boules M, Lin SC, Richelson E. Neurobiologic basis of nicotine addiction and psychostimulant abuse: a role for neurotensin? Psychiatr Clin North Am 2005;28(737–51):746.
Gewirtz JC, McNish KA, Davis M. Is the hippocampus necessary for contextual fear conditioning? Behav Brain Res 2000;110:83–95.
Goldstein BJ, Goodnick PJ. Selective serotonin reuptake inhibitors in the treatment of affective disorders—III. Tolerability, safety and pharmacoeconomics. J Psychopharmacol 1998;12:S55–87.
Goodnick PJ, Goldstein BJ. Selective serotonin reuptake inhibitors in affective disorders—II. Efficacy and quality of life. J Psychopharmacol 1998;12:S21–54.
Kalivas PW, Nemeroff CB, Prange Jr AJ. Neurotensin microinjection into the nucleus accumbens antagonizes dopamine-induced increase in locomotion and rearing. Neuroscience 1984;11:919–30.
Kanba KS, Kanba S, Nelson A, Okazaki H, Richelson E. [3H]neurotensin(8–13) binds in human brain to the same sites as does [3H]neurotensin but with higher affinity. J Neurochem 1988;50:131–7.
Kehne JH, Casella JV, Davis M. Anxiolytic effects of busirone and gepirone in the fear-potentiated startle paradigm. Psychopharmacology (Berl) 1988;94(1):8–13.
Lambert PD, Ely TD, Gross RE, Kilts CD. Neurotensin induces Fos and Zif268 expression in limbic nuclei of the rat brain. Neuroscience 1996;75:1141–51.
LeDoux JE. Emotion circuits in the brain. Annu Rev Neurosci 2000;23:155–84.
Machida R, Tokumura T, Tsuchiya Y, Sasaki A, Abe K. Pharmacokinetics of novel hexapeptides with neurotensin activity in rats. Biol Pharm Bull 1993;16:43–7.
Mansbach RS, Geyer MA. Blockade of potentiated startle responding in rats by 5-hydroxytryptamine1A receptor ligands. Eur J Pharmacol 1988;156(3):375–83.
Mansbach RS, Geyer MA, Braff DL. Dopaminergic stimulation disrupts sensorimotor gating in the rat. Psychopharmacology 1988;94:507–14.
Milham MP, Nugent AC, Drevets WC, et al. Selective reduction in amygdala volume in pediatric anxiety disorders: a voxel-based morphometry investigation. Biol Psychiatry 2005;57:961–6.
Mora PO, Netto CF, Graeff FG. Role of 5-HT2A and 5-HT2C receptor subtypes in the two types of fear generated by the elevated T-maze. Pharmacol Biochem Behav 1997;58:1051–7.
Myers K, Goulet M, Rusche J, Boismenu R, Davis M. Inhibition of fear potentiated startle in rats following peripheral administration of secretin. Psychopharmacology (Berl) 2004;172:94–9.
Nakachi N, Yamada M, Cho T, Coleman NJ, Yamada M, Richelson E. Expression of mRNA for neurotensin in rat brain cholinergic neurons. Biol Psychiatry 1995;37:642.
Paxinos G, Watson C. The rat brain in stereotaxic coordinates. New York, New York: Academic Press; 1997.
Petrie KA, Bubser M, Casey CD, Davis MD, Roth BL, Deutch AY. The neurotensin agonist PD149163 increases Fos expression in the prefrontal cortex of the rat. Neuropsychopharmacology 2004;29:1878–88.
Richelson E, Fredrickson PA, Boules MM. Neurotensin receptor agonists and antagonists for schizophrenia. Am J Psychiatry 2005;162:633–4 author reply 635.
Rickels K, Rynn M. Pharmacotherapy of generalized anxiety disorder. J Clin Psychiatry 2002;63(Suppl 14):9–16.
Robledo P, Maldonado R, Koob GF. Neurotensin injected into the nucleus accumbens blocks the psychostimulant effects of cocaine but does not attenuate cocaine self-administration in the rat. Brain Res 1993;622:105–12.
Saiz Ruiz J, Carrasco Perera JL, Hernanz A. [Plasma neuropeptides in affective and anxiety disorders]. Arch Neurobiol (Madr) 1992;55:1–5.
Shilling PD, Richelson E, Feifel D. NT69L, a neurotensin agonist, produces antipsychotic-like effects on prepulse inhibition of startle. Behav Brain Res 2003;143:7–14.
Shilling PD, Melendez G, Priebe K, Richelson E, Feifel D. Neurotensin agonists block the prepulse inhibition deficits produced by a 5-HT2A and an alpha1 agonist. Psychopharmacology (Berl) 2004;175:353–9.
Shugalev IP, Ol'shanskii AS, Lenard L, Hartmann G. [Effects of neurotensin on active and passive avoidance performance in rats with lesions of serotoninergic neurons]. Zh Vyssh Nerv Deiat Im I P Pavlova 2005;55:247–52.
Skoog KM, Cain ST, Nemeroff CB. Centrally administered neurotensin suppresses locomotor hyperactivity induced by D-amphetamine but not by scopolamine or caffeine. Neuropharmacology 1986;25:777–82.
Szigethy E, Beaudet A. Selective association of neurotensin receptors with cholinergic neurons in the rat basal forebrain. Neurosci Lett 1987;83:47–52.
Walker DL, Davis M. The role of amygdala glutamate receptors in fear learning, fear-potentiated startle, and extinction. Pharmacol Biochem Behav 2002;71:379–92.
Weisstaub NV, Zhou M, Lira A, et al. Cortical 5-HT2A receptor signaling modulates anxiety-like behaviors in mice. Science 2006;313:536–40.
Winslow JT, Noble PL, Davis M. Modulation of fear-potentiated startle and vocalizations in juvenile rhesus monkeys by morphine, diazepam, and buspirone. Biol Psychiatry 2007;61(3):389–95.
Wustrow DJ, Davis MD, Akunne HC, et al. Reduced amide bond neurotensin 8–13 mimetics with potent in vivo activity. Bioorg Med Chem Lett 1995;5:997–1002.
Yamauchi R, Wada E, Yamada D, Yoshikawa M, Wada K. Effect of beta-lactotensin on acute stress and fear memory. Peptides 2006;27:3176–82.